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THIRTY YEARS' EXPERIENCE WITH ORCHARD SPRAYS IN NOVA SCOTIA[1]

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During thirty years, orchard practices, particularly those relating to the control of pests, have changed materially. The gradual evolution of spray technique in Nova Scotia is probably roughly paralleled by similar developments in other orchard districts, yet it was thought that a brief outline of spray procedure during the period in question would be of interest and worthy of record.

The principal factors influencing spray practices have been the accumulated experiences of growers, the spread of knowledge concerning procedures in other regions, and the experiments and investigations of the Provincial and Dominion Government services. In this paper no attempt has been made to give credit to, or fix the responsibility upon, any party or parties for the various changes made, but merely to record the practices of the period in so far as they have come within the experience of the writer.

The first time the writer wielded a spray rod was in 1908, and it must be admitted that, in company with a good many growers at that time, it was with doubt as to whether a scientific operation was being performed, or whether an over-credulous disposition had led one into an act of foolishness. Spraying was a fairly general practice in 1908 among the more progressive men, but was by no means practised by all commercial apple growers. In fact, the merits or demerits of spraying was a common source of discussion and argument. It was conceded by all that Paris green would destroy caterpillars, but, as to the chemical control of apple scab, that was another question. If materials sufficiently toxic to destroy fungi were employed, would not the trees also be poisoned, the soil become sterile, the birds killed? Nevertheless, within the next year or two, spraying was a routine procedure for nearly all growers.

For the promotion of better orcharding, it was the practice of the Provincial Department to engage a few of the more up-to-date growers to give lectures at various points through the fruit-growing districts. Frequent topics were: the preparation of Bordeaux mixture, the making of sodium arsenite, and the control of apple scab, codling moth (*Carpocapsa pomonella* L.), budmoth (*Spilonota ocellana* D. & S.), and cankerworms (*Also-*

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phila pometeria Harr.). The eye-spotted budmoth (*Spilonota ocellana* D. & S.) was a serious pest of Nova Scotia orchards, just as it is to-day. Judging from the limited number of orchards under the observation of the writer in the years immediately following 1908, codling moth (*Carpocapsa pomonella* L.) is less today in the same area than it was then.

The spray used was Bordeaux mixture composed of equal weights of copper sulphate and lime, with which was incorporated either Paris green or sodium arsenite. The Bordeaux mixture was made by the old stock solution method, that is, concentrated solutions or suspensions were prepared in advance, and diluted and mixed as required. Many growers prepared a stock solution of sodium arsenite from white arsenic and sodium carbonate. Hand pumps were, of course, used for spraying, and four applications were made during the season: two pre-blossom and two post-blossom.

On the whole, fairly good results were obtained in the control of apple scab. Cankerworms (*Alsophila pometaria* Harr.) and tent caterpillars (*Malacosoma americana* Fab., and *Malacosoma distria* Hb.), which were a great scourge of orchards, were brought within subjection, and codling moth (*Carpocapsa pomonella* L.) declined in numbers. However, on some varieties of apples russeting was produced by the Bordeaux mixture, though the russeting from the light sprays put on by hand pumps was by no means as severe as that which would be anticipated today if the same mixture were used and applied with our modern apparatus and at our present gallonage. In addition to russeting of fruit, orchards were frequently observed which carried a considerable amount of "yellow leaf", this latter condition developing mostly in late summer. These defects in fruit and foliage were generally, and rightly so, attributed to the Bordeaux mixture. However, looking back it seems not improbable that some of the yellow leaf may have been caused from excessive dosages of sodium arsenite, for this poison, while quite compatible with Bordeaux mixture in small dosages, is not so when used above certain limits. Nevertheless, in spite of the defects of the spray mixture, it was obviously profitable to spray with it.

The writer's first experience with lime-sulphur as a summer spray was in 1910, although it had been used previously by a few growers. This material was generally hailed with satisfaction for it controlled apple scab and at the same time did not cause the types of injury to which growers had become accustomed from the formula previously used. For the next few years lime-sulphur rapidly replaced Bordeaux mixture as a summer spray, but never did so entirely. There were always some growers who continued to use Bordeaux and who claimed that, all things considered, it was preferable. About the same time that lime-sulphur was introduced, lead arsenate came into use, being made on the farm by mixing together solutions of lead acetate and sodium arsenate, though later the use of commercial lead arsenate paste became general. During the same period also, power sprayers were introduced, and spraying was done on a scale not heretofore attempted.

However, while the introduction of the lime-sulphur lead arsenate spray was generally regarded with satisfaction, almost from the start some growers claimed that the material was dwarfing the leaves and decreasing

the yield. After a few years, this idea of lower yield became prevalent. The writer recalls an occasion in 1914 when he inspected with considerable curiosity a row of unsprayed Gravenstein trees loaded with scabby apples and an adjoining row sprayed with lime-sulphur carrying a light crop of perfectly clean fruit. Observations and experiments during 1915 and 1916, corroborated the common expression, "The apples were sprayed off the trees." However, in the writer's opinion this crop reduction was considerably exaggerated since many growers blamed the spray material for low yields quite irrespective of other factors.

In the meantime developments along other lines had been taking place. Some growers had been privately testing the efficiency of sprays composed of a heavy lime whitewash. Finding that this did not control scab, they had added to the whitewash small amounts of copper sulphate. Work along this line commenced in 1913, and shortly thereafter attracted considerable attention. These spray mixtures, consisting of small amounts of copper sulphate combined with large excesses of lime, controlled apple scab and maintained the trees in excellent foliage, although russeting of apples was present to some extent. In the years following and particularly in 1917, experiments were conducted with Bordeaux mixture of varying copper content and with various ratios of copper to lime. It was shown that, at least under some conditions, lime in considerable excess of previously used formulae was distinctly advantageous in reducing copper injury to foliage. Coincidentally, or shortly thereafter, it was also shown that in a four-spray schedule, the russeting of apples was caused almost entirely by the immediate pre-blossom and post-blossom sprays. These findings had a great influence on the future development of spray calendars in Nova Scotia.

During this period, namely 1914 to 1918, calcium arsenate came into use replacing lead arsenate to a considerable extent. This was done at a time when lead was high in price, but the use of calcium arsenate persisted as it does to this day. While it was found quite unsuitable for use alone on apple foliage, it was found compatible and effective in Bordeaux mixture and also in several of the sulphur sprays under certain conditions.

Sodium polysulphide was also used considerably during this period and for several years later. This material was generally condemned as a summer spray in other orchard regions, probably because it was incompatible with lead arsenate. In Nova Scotia it was used with calcium arsenate and hydrated lime, and, while the fungicidal value was weak at the dosages used, little or no foliage injuries resulted. The sodium polysulphide had the further advantage of being compatible with soaps and could be combined effectively in this combination with nicotine sulphate and used successfully against aphids and the green apple bug (*Lygus communis* Knight). The practice of using nicotine against these insects became general at about this time.

The extensive practice of orchard dusting commenced about 1918, although of course, some dusting had been done prior to this. Orchard dusting developed rapidly and large numbers of growers began to rely entirely on the dust method of control. This practice came to its height about 1924, after which it gradually declined until at the present time dusting is done by only a few, and this mostly as an adjunct to spraying. There

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were several reasons for the rise in the practice of dusting. One was the realization, and perhaps exaggeration, of the defects of the spray materials as then generally known. At this period also, many growers who had increasing acreages of bearing orchard and were finding it difficult or impossible to spray with the apparatus then available, could successfully treat the orchard with dust on account of the shorter time required.

The dusts used consisted of mixtures of finely ground sulphur and lead arsenate, generally in the ratio of 90 to 10, or of Bordeaux dust, commonly called copper-lime dust. The latter had been developed in Nova Scotia in 1917 and 1918, and consisted of mixtures of hydrated lime, dehydrated copper sulphate, and calcium arsenate. Most growers were able to control apple scab fairly well with the dust procedure, but a few found it difficult to do so. Nicotine dust, that is, hydrated lime impregnated with nicotine sulphate was also widely used during the same period. Dusting with this combination against the green apple bug (*Lygus communis* Knight), was eminently successful.

This period of seven or eight years during which dusts were so extensively used was terminated mainly by two factors. Extreme outbreaks of the eye-spotted budmoth (*Spilonota ocellana* D. & S.) occurred in the years between 1924 and 1927, causing great damage to the crops. These could not be controlled by any known dusting method. At the same time, in a large number of orchards, the European red mite (*Paratetranychus pilosus* C. & F.) became a serious pest and this likewise was not amenable to dust treatment. These two factors coupled with the development of improved high powered spray outfits, lead to a decline in the dust method of control and the revival of the spray system.

The spray calendar followed during the 1920's was based, for the most part, on Bordeaux mixture containing excess lime, with a wettable sulphur spray used on the calyx application. A few growers, but only a few, used the lime-sulphur spray. Calcium arsenate was used with the Bordeaux mixture and lead arsenate with the wettable sulphur and lime-sulphur. As a rule, four applications were made, but towards the latter part of the period it became common to make three pre-blossom applications in orchards where scab was difficult to control. In some orchards infested with either budmoth (*Spilonota ocellana* D. & S.), or the gray-banded leaf roller (*Eulia mariana* Fern.), it became necessary to apply three post-blossom applications. Thus growers were making from four to six applications. As a rule, the larger number of applications was found profitable so that five or six applications became customary. At the present time six applications is the general rule.

The severe outbreak of eye-spotted budmoth (*Spilonota ocellana* D. & S.) was followed during the latter part of the 1920's by a more or less severe outbreak of the gray-banded leaf roller (*Eulia mariana* Fern.). The latter has remained a major economic pest ever since. These two pests had a considerable influence on spray technique. Both require very careful and thorough work in order to obtain adequate control. Foliage has to be wet on the underside with either nicotine sulphate or an arsenical or both, and the necessity of controlling these pests led many growers into the habit of using a greater gallonage of spray material and to an improvement in the type of work done.

The use of mineral oil sprays in the dormant or delayed dormant periods, likewise was commenced mostly in the latter part of the 1920's. At first this was directed primarily against the European red mite (*Paratetranychus pilosus* C. & F.), but as time went on the successful use of oil sprays was developed against oyster shell scale (*Lepidosaphes ulmi* L.), the apple mealy bug (*Phenacoccus aceris* Sig.) and the buffalo tree hopper (*Ceresa bubalus* Fab.). Coal tar oils likewise were experimented with during the same period, but in the following years were but little used. They are used to a small extent at the present time against aphids and the apple mealy bug (*Phenacoccus aceris* Sig.).

In 1924 an extensive series of experiments was commenced with lime-sulphur precipitated with the sulphates of copper, zinc, aluminium, and iron, these mixtures being combined with various arsenicals and other insecticides. As a result, a mixture of lime-sulphur, aluminium sulphate, and calcium arsenate, was tentatively recommended for growers' use. However, in 1929 a mixture of lime-sulphur, iron sulphate, and calcium arsenate was recommended, and this was widely adopted and has been in continuous use up to the present time. This mixture gave control of apple scab and biting insects essentially the same as that from the lime-sulphur lead arsenate mixture, but without the defects of the latter. The colour of the mixture is objectionable, and its spreading properties are not as good as might be desired. Nevertheless, it served, and continues to serve, a useful purpose.

During the last fifteen years there have been under continual trial by both growers and experimenters, all the various types of wettable and colloidal sulphurs, and other sulphur compounds such as calcium monosulphide, which have been offered on the market, together with several others devised locally. For the most part, arsenicals used with these materials gave excellent control of biting insects. Most of these materials were not sufficiently fungicidal for Nova Scotia conditions, although they would do very well when they were both preceded and followed by Bordeaux mixture. Within recent years, however, the use of flotation sulphur of the ferrox type has increased with great rapidity, since it was found to be strongly fungicidal in practical dosages. During 1938 this material has, to a considerable extent, replaced the iron sulphate lime-sulphur mixture.

During the last few years such insecticides as the fixed nicotines, cryolite, phenothiazine, derris, and pyrethrum, have all been under more or less extensive investigation both as to their use in the regular spray schedules and as to their use for special purposes. As a result of this work pyrethrum has come into practical use, both in spray and dust form, for combating the green apple bug (*Lygus communis* Knight) and the apple red bug (*Lygidea mendax* Reut.). The other insecticides mentioned have not, as yet, been adopted in practice, though there are prospects that one or two of them may be used for special purposes.

Summarizing present spray practices, a six-spray schedule is followed, the first two and the last consisting of an excess lime Bordeaux combined with calcium arsenate. The three intermediate sprays are the iron sulphate lime-sulphur mixture combined with calcium arsenate, or flotation sulphur and lead arsenate. Of course, there are many variations from the above, and wettable sulphurs of various types are also used, but mostly

as intermediate sprays being preceded and followed by Bordeaux mixture. Coal tar oils are used in the dormant season, but only to a small extent, against aphids and the apple mealy bug (*Phenacoccus aceris* Sig.). Mineral oils are used in the dormant or delayed dormant period, but only when and if required for special purposes. The use of oil sprays is discouraged except under conditions of necessity. While these are useful insecticides, their after-effects are undesirable as they appear to raise havoc with the natural control of the European red mite (*Paratetranychus pilosus* C. & F.). Nicotine sulphate is used in the delayed dormant period to control aphids, and later, when required, to control apple sucker (*Psyllia mali* Schmid.), budmoth (*Spilonota ocellana* D. & S.), gray-banded leaf roller (*Eulia mariana* Fern.), and several other insects. Pyrethrum is used to combat the green apple bug (*Lygus communis* Knight) and the apple red bug (*Lygidea mendax* Reut.).

This is just a brief outline of some of the developments which have occurred within the past thirty years, and which have led up to the schedules at present followed.

FURTHER NOTES ON THE FALL CANKERWORM AND ITS CONTROL BY "SOLID-STREAM" SPRAYING

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Some notes have already been published on an outbreak of the fall cankerworm (*Alsophila pometaria* Harris) which has occurred on the large elms in Fredericton, N.B. (1). These notes had particular reference to the effectiveness and difficulties of banding. Since then further investigations have been conducted, and the outbreak in the city has been completely controlled by spraying, although it continues in the woodlands of the St. John valley. This paper gives additional notes on the bionomics of the insect and describes the methods of control by spraying. Although there are many references to this species in the literature, most of them refer to conditions farther south and to control in orchards. There is, apparently, no detailed account of an extensive control operation on shade trees.

During the above outbreak no evidence has been found of the presence of the spring cankerworm (*Paleacrita vernata* Peck) which is generally associated with the fall cankerworm in the United States.

NOTES ON BIONOMICS

Eggs

The eggs are laid in compact masses on the bark of the stem and branches, but most of them have been found near the end of the smaller branchlets of the favoured hosts. They may also be placed on nearby upright objects such as fences, telephone poles, etc. The masses vary in size from a very few to over 200. Most females lay all their eggs in one mass and the average size of 37 masses collected was 148. They are laid during the cool weather of November and no development takes place until the following spring.

An experiment was carried out to determine whether the eggs would hatch without a period of conditioning by exposure to low temperatures. One lot of eggs was obtained by oviposition in a chamber at 72 ± 2° F. and these were kept at this temperature for 85 days and then examined. Another much larger lot was collected and left outside, a sample being brought into the laboratory every week and incubated at 72° F., relative humidity 80, for 35 days. They were then examined under a binocular microscope. The eggs were collected at random from scattered trees. Most of them were laid around November 6. Results are shown in Table 1.

It will be seen that of the lot which experienced no temperatures below the threshold of development, 90% eventually formed embryos and 72% hatched. Exposure to low temperatures is not therefore essential to development but in this case mortality prior to hatching was rather high and no eggs hatched until after 45 days.

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Several workers have shown that certain hibernating stages experience a period of quiescence regardless of temperature but that previous exposure to low temperatures shortens this period after they are submitted to incubation above the threshold of development. Bodine (2) demonstrated this to be the case with certain grasshopper eggs. Fink (6) showed that

TABLE 1.—EGGS BROUGHT IN AT WEEKLY PERIODS AND INCUBATED AT 72° F. FOR 35 DAYS, THEN EXAMINED (WINTER 1937–38)

Date brought in	Min. temp. °F.	Days to first hatch	Total eggs	Percentage						Per cent development (excl. parasitized)
				Hatched	Embryos		Parasitized	Undeveloped eggs		
					Living	Dead		Living	Dead	
*Nov. 10	71.0	45	1340	72.0	0.4	15.4	0	1.0	11.2	89.8
Dec. 6	12.5	—	1863	21.5	42.7	0	3.7	30.3	1.8	66.6
13	7.0	25	2741	30.3	45.4	0	0.9	21.4	2.0	76.3
20	7.0	24	2796	48.3	43.0	0	3.8	3.9	1.0	94.9
27	9.8	21	2280	55.9	27.5	0	11.7	2.6	2.3	94.4
Jan. 4	−21.8	20	4169	70.0	20.8	0	5.5	2.0	1.7	96.0
10	−21.8	18	3753	67.8	21.2	0	8.4	2.0	0.6	97.1
17	−21.8	18	3624	76.4	9.9	1.6	10.5	0.7	0.9	98.2
24	−21.8	17	3863	72.7	11.2	8.1	7.4	0.3	0.3	99.3
31	−21.8	16	3182	72.1	1.3	12.5	12.9	0.3	0.9	98.6
Feb. 7	−21.8	13	3943	75.5	0.3	14.1	9.0	0.5	0.6	98.7
14	−21.8	12	2740	71.7	0	14.1	13.4	0.4	0.4	99.0
21	−21.8	13	2976	81.2	0	5.5	12.3	0.5	0.5	98.8
28	−21.8	12	2638	90.6	0	3.6	4.3	0.5	1.0	98.4
Mar. 8	−24°	12	2389	88.0	0	2.6	7.4	0.7	1.3	97.9
14	−24°	12	2351	83.4	0	2.0	12.7	0.4	1.5	96.8
21	−24°	11	2465	84.7	0	0.7	11.8	2.2	0.6	96.8

* Eggs laid in chamber at 72° F., examined after 85 days.

the hibernating adult of the potato beetle, and Bodine and Evans (3) that the larva of the mud-dauber wasp, also go through a similar so-called "recovery period". Flemion and Hartzell (7) showed that the eggs of *Malacosoma dissatria* would not hatch without exposure to low temperature and that the period of incubation was similarly shortened by exposure to cold. They reported somewhat similar results with *Alsophila pometaria*, but a few hatched without being placed outside.

In Table 1 it will be seen that as the time of exposure to outside winter temperatures was increased the length of time which it took the first eggs to hatch decreased, as did also the percentage which died in the course of development. Of the eggs which, for instance, experienced about 44 days of low temperature, 24% had not developed after five weeks at 72° F. On the other hand, almost all of the eggs left outside for 51 days or more began to develop within the 5 weeks although an exposure of some 65 days to low temperatures was apparently necessary to obtain 100% development within this time.

It is evident from the lots brought in on December 6 and 13 that the eggs of the cankerworm when not conditioned by exposure to winter tem-

peratures undergo a period of diapause, or "recovery" before development commences. This recovery period, however, is shortened by a pre-incubation exposure to low temperatures. The longer this exposure, the quicker the recovery from the hibernating condition although the effect is not much increased with exposures of over 86 days. It would appear that after approximately 3 months the factors making for diapause (Bodine's "x factors") are almost completely inhibited.

The time of hatching is important in timing spraying operations. As the position of the eggs in relation to the sun and its direct or reflected heat influences the rate of incubation, there is sometimes a considerable period between the commencement and completion of hatching. It is necessary to mark a number of masses in different positions on the trees, particularly on branches well above the ground and on the north side of the trunks, and observe them daily. In warm weather the eggs of a single mass often hatch almost simultaneously and the hatching period for the whole population is shorter than in cool weather. Most of the eggs tend to hatch within a few days after the leaf buds of the elm trees have all burst. The year following complete defoliation, however, trees are slow in putting out leaves, and eggs may hatch before the buds are fully open.

During five different seasons the first eggs hatched on May 11, 12, 13, 14 and 17. The last eggs hatched on May 25, 20, 24, 21 and 27 in the same respective years, giving a hatching period of from 7 to 14 days.

The percentage of hatch has been determined in the same years after eliminating parasitized eggs. It has ranged from 96.0 to 98.5%, with the exception of 1934, when it was estimated at 89.5% following a minimum winter temperature of −31° F. The number of infertile eggs has therefore been small, as is also indicated by Table 1. It has been found that unfertilized females seldom lay eggs. Porter and Alden (6) report much higher percentages of infertile eggs, almost 50%. This may be associated with the unusually low percentage of males which they noted.

Larvae

On hatching, many larvae drop on a thread of silk from a point near the egg mass, regardless of the proximity of foliage. This has been observed under field conditions and in the laboratory. In the latter case almost half the newly emerged larvae dropped from the twigs to which their eggs were attached, within 15 minutes. A comparatively slight air movement will carry such larvae in an almost horizontal direction and this is undoubtedly the chief method of dissemination (1). After feeding has commenced few larvae drop in this way unless defoliation is severe, when on a warm day thousands can be seen hanging from the trees on long threads.

The young larvae feed on the under sides of the leaves after they have unfolded and are fairly well protected from rain. They can survive a certain amount of cold, wet weather. On May 15, 1937, the temperature dropped to 34° F. and there was a fall of rain and some snow. The following morning a considerable number of newly hatched larvae were seen to be covered with water. The twigs were brought into the laboratory and the great majority of the larvae became active and began feeding. Some of the smaller ones died.

The first sign of feeding consists of small holes in the expanding young leaves. Later, if the larvae are numerous, all of the leaf may be eaten except the main veins. The leaves toward the ends of the branches are the first to show serious damage.

The favoured hosts have been elm, basswood, apple, soft maple and oak, in approximate order of preference. Butternut, ash, poplar and alder have also been fed on freely in woodlands. Hard maple was seldom much injured. Comparative feeding experiments with 4 lots of 35 third-stage larvae on elm, oak, red maple and hard maple gave a survival to the pre-pupal stage of 33, 30, 13 and 6, respectively, and the larvae were very restless on the hard maple which was evidently unsatisfactory as food. All these larvae, however, were taken from elm.

Twenty-five larvae have been reared individually from the egg and all had 4 instars. Both sexes were included.

Some larvae are green with light stripes; others have dark brown to black stripes and markings which may predominate over the green. Both phases are represented in both sexes.

The larvae mature around the middle of June, about a month after hatching, and commence to drop to the ground where they enter the soil and form an oval cell lined with a thin but rather tough cocoon. Counts of these cocoons have been made from 22 square-foot quadrats from which the cocoons were removed in 1-inch layers. Some quadrats were in lawns, and others in comparatively loose soil under shrubs.

Depth	*Lawn*	*"Loose" soil*
1″	730 cocoons	36 cocoons
2″	131 cocoons	218 cocoons
3″	20 cocoons	20 cocoons
4″	0 cocoons	5 cocoons

An additional 28 quadrats were taken in sod and loose or weedy soil. A slight preference was shown by the larvae for the looser soil. The highest number of sound cocoons per square foot, namely 76, was found, however, in a lawn. Any undisturbed garden soil or lawn is suitable for spinning but no attempt is made to enter surfaces covered with cinders or gravel. The susceptibility of a city area to severe defoliation is determined partly by the amount of paving, and partly by the concentration of the favoured hosts. Complete defoliation has not occurred where more than about 60% of the ground beneath the trees was paved.

Pupation takes place in from 3 to 4 weeks after the cocoon is formed and the pupae remain in diapause until the fall.

Adults

Emergence of the adults from the ground does not generally take place until after the first frost, although it is doubtful if frost is in any way necessary to emergence. It does, however, seem to stimulate emergence, as large numbers generally come out of the ground following frosty nights if the day is warm. The males emerge 2 or 3 days earlier than the females. The earliest record of male emergence was October 12, the earliest female emergence October 14, and the latest November 22. Two elms in the heavily infested area were double-banded in 1936, 1937 and 1938, and the

females beneath the bands collected and counted every day or so. This work had to be discontinued before emergence was over in 1936. In 1938, these trees were sprayed and almost 100% control obtained. Three other trees in an unsprayed, less heavily infested area were banded in that year as a comparison. The results are shown in Table 2.

It will be seen that the period of emergence may last from October 15 to November 22 and the peak occurs around November 3 to 5. The highest emergence in one day occurred on November 3, in 1937, a warm day following frost, when from $\frac{1}{3}$ to over $\frac{1}{2}$ of the total emergence took place and 4,631 females climbed one tree. The largest number to climb one tree in one season was 6,823. Judging from the facts that in 1937 all the foliage was destroyed when the larvae were not much more than half grown and the females were undoubtedly twice as numerous in 1938, it may be roughly

TABLE 2.—FEMALES TAKEN BENEATH BANDS

Date	Tree No. 1 (diameter 26″)			Tree No. 2 (diameter 15″)			Unsprayed trees,
	1936	1937	1938	1936	1937	1938	1938
Oct. 15	7	–	–	10	–	–	–
16	–	2	–	–	2	0	0
17	12	–	–	24	–	–	3
18	–	–	–	–	–	–	6
19	–	4	–	–	1	0	–
20	10	–	–	15	–	–	10
21	18	4	–	85	23	0	–
22	–	–	–	–	–	–	12
23	–	23	–	–	96	–	98
24	46	–	–	136	–	0	–
25	–	31	–	–	100	–	–
26	60	40	1	107	180	1	36
27	–	87	–	–	481	0	–
28	–	48	1	–	163	0	102
29	–	42	–	–	132	0	80
30	–	–	–	–	–	–	–
31	281	–	–	502	–	0	221
Nov. 1	–	78	–	–	97	0	87
2	396	–	–	675	–	–	416
3	–	1167	–	–	4631	0	143
4	314	184	–	602	164	–	159
5	*	257	–	*	300	–	670
6	*	–	–	*	–	0	–
7	*	–	–	*	–	–	511
8	*	283	2	*	170	0	254
9	*	126	–	*	85	–	–
10	*	113	–	*	80	–	174
11	*	–	–	*	–	0	–
12	*	12	–	*	5	0	212
13	*	–	–	*	–	0	–
14	*	–	–	*	–	–	173
15	*	–	–	*	–	–	–
16	*	306	–	*	71	0	–
17	*	100	–	*	28	–	34
18	*	–	–	*	–	–	Snow
19	*	81	–	*	14	–	–
20	*	–	–	*	–	–	115
22	*	5	–	*	0	–	13
	1144	2993	4	2156	6823	1	3523

* Discontinued.

estimated that when over 1,000 females ascend an average elm tree of 24 inches diameter, it is in danger of practically complete defoliation. Comparisons between the early feeding in 1937 and before spraying in 1938 suggest that there were probably 4 times as many larvae on these trees in 1938 as would have sufficed to strip them of foliage.

The males are most active in the evening for about two hours after sundown but some fly in the daytime. They can sometimes be seen in thousands fluttering over the ground beneath the trees or about the bases of the trunks until the ground appears to quiver. Mating takes place shortly after emergence, on the ground or on the trunks. Both sexes are active at remarkably low temperatures. A male was observed in flight in an air temperature of 34° F. at 4:30 p.m. when the sun was shining, and mating with movement has been observed at 38° F. without sunshine.

The sex ratio has been close to 1 : 1 each year. Of 1,710 emergences recorded from cages in which field-collected larvae were reared, 903 were females, 807 males. This contrasts with Porter and Alden's report of a ratio in Connecticut of 2 males to 151 females in 1919 and 1 : 385 in 1920. Riley (10) also obtained only 2 males out of 58 reared specimens in Missouri.

Of 14 females reared from the cocoon in cages without mating one laid 11 and another 2 eggs. These did not develop. A very low percentage of males would seem to be likely to reduce the reproductive rate considerably, although males have been found capable of mating several times. At Fredericton, very few females failed to mate.

Unmated females kept in vials have remained without laying any eggs for up to 10 days and then oviposited within a few hours after being mated. The preoviposition period may be from 1 to 10 or more days, depending on the temperature and time of mating, but most females oviposit within 3 or 4 days of emergence. The length of life of 19 females reared in the insectary ranged from 10 to 27 days and averaged 18 days. Oviposition was observed at 37° F.

The number of eggs laid by a female is dependent partly on the feeding history of the larva from which it developed and partly on the time of

TABLE 3.—OVIPOSITION

Lot	No. of females	Size	Treatment	Average eggs per female		
				Laid	Remaining in abdomen	Total
1	13	Average	Placed in insectary.	83	58	141
2	6	Average	Placed in insectary. Removed to laboratory November 13.	104	47	151
3	14	Average	Placed in insectary, unmated.	1	170	171
4	19	Average	Constant temperature 72° F.	80	44	124
5	60	Small	Collected under completely defoliated trees, placed in insectary.	?	?	59
6	20	Small	Collected under completely defoliated trees, placed in insectary.	44	8	52
7	17	Medium	Collected under completely defoliated trees, placed in insectary.	78	21	99
8	15	Large	Collected under partially defoliated trees.	79	111	180

emergence and mating. A number of adults were placed singly in vials in the insectary, each with a male, and allowed to oviposit on blotting paper. After death they were dissected and the number of eggs in the abdomen was determined. The results are shown in Table 3.

Lots 5 to 7 were collected in 1937 in an area where defoliation had been complete, to determine what effect the partial starvation had on oviposition. It was found that the egg capacity of the small females was reduced to about $\frac{1}{3}$ of the average but they laid a higher percentage of their eggs than the females of more normal size.

THE OUTBREAK

The Fredericton outbreak is one of a number which have been reported during the past 6 years from many points in North America, including California, Kansas, Indiana, Manitoba, Ontario, New York, and most of the New England states. It affords another instance of the fact that the epidemiology of many insects is governed by factors which are continent-wide in their influence. In New Brunswick and Nova Scotia, the insect has been present in unusual numbers from 1931 to 1938 but severe defoliation has resulted only in the lower St. John valley and in Annapolis, Kings and Queens counties, Nova Scotia. Defoliated areas have been characterized by a preponderance of either oak or elm.

The severely infested part of the St. John valley extended from Fredericton to Jemseg, a distance of 27 miles, where considerable areas of elm woodlands occur. In 1931, males were found to be numerous. From 1932 to 1936, defoliation was noticeable and showed a gradual increase, except in 1934 when the attack was less severe than in 1933. During this time a few trees lost up to 90% of their foliage. In 1937, the insect increased sufficiently to defoliate completely a large number of trees in the city and woodlands. The trees in the southeastern half of the city were plotted on a map in 3 degrees of defoliation: 406 large elms were stripped, 358 lost over one-half their foliage, and 186 in the same area lost less than one-half. These trees ranged in diameter from 18 to 50 inches, and were from 50 to 100 feet in height. In addition, 122 smaller trees were stripped and 106 lost over one-half their foliage. In other parts of the city defoliation varied from 10 to 30%. The severe damage occurred where the elms were most numerous and where not more than about 60% of the ground beneath the trees was paved.

Most of the completely defoliated trees lost all their leaves before the larvae were full grown, and the partially fed larvae descended in great numbers on the gardens and streets, causing great annoyance for about two weeks. Seedlings were destroyed and many shrubs, especially lilac and rose bushes, were stripped. Sidewalks were covered with the dead caterpillars, which caused an unpleasant odour. Torches, brooms and spray guns were in frequent use to combat the migrating larvae.

Large numbers were killed or failed to mature through scarcity of food, and in order to get some idea of the effect this might have had on the population a few samples of the cocoons were taken in July from quadrats of 1 square foot, placed at random beneath trees. The results are shown

in Table 4. Owing to the fact that old cocoons are difficult to identify, only the figures for cocoons showing living canker worms or parasites are presented. The earth, taken to a depth of 4 inches, was passed through a sieve to prevent cocoons being missed.

TABLE 4.—COCOON SAMPLES

Date taken	Defoliation, %	No. of quadrats	Av. No. cocoons per sq. ft.	
			Sound	Parasitized
July, 1937	22 – 55	10	13.5	0.3
" "	85 – 100	13	13.0	0.2
Sept., 1937	75 – 100	28	16.1	0.3
Oct., 1938	0 (sprayed)	18	0.5	0.1
" "	50 – 75 (unsprayed medium infestation)	13	5.0	0

The figures showed that there were many cocoons in the ground even where starvation was severe. A further count at the same points was made in September to determine if a high mortality had resulted during the summer owing to poor feeding conditions, but it will be seen that the number per square foot was even higher although samples were too few for significant comparison. Counts of the adults ascending trees in the fall of 1937 (Table 2) and observations of the prevalence of egg masses increased the expectation of a heavier attack in 1938, and observations on the flight of the male moths indicated that the damage would be more widespread (1).

As the result of spraying, the outbreak in the city was interrupted in 1938 but the numbers of the young larvae on the trees and the amount of feeding before spraying clearly indicated that the insect was more numerous and would have completely defoliated a considerably larger number of trees than in 1937. This was confirmed by observations in the unsprayed woodlands outside the city where a majority of the trees were stripped, some for the first time, and even isolated trees on neighbouring farm land lost up to 75% of their foliage.

Observations on the moths in October, 1938, indicate that the outbreak will continue in 1939. No special study of control factors has been possible but it is evident that parasites have not been very numerous at any time. One larval parasite, *Macroctenrus amicroploides* Vier., was reared. In no case did any collection of larvae or cocoons show a total parasitism in excess of 5%.

Trichogramma sp. and another unidentified Chalcid were found attacking the eggs each fall. In Table 1 it will be seen that parasitism of eggs collected in 1937 ranged from 1 to 13%. A lot of 24 masses, containing 3637 eggs was collected in April, 1937, and on rearing found to be 33% parasitized. In February, 1938, another lot of 14,864 was collected and 8% found to be parasitized.

Parasitism seems, therefore, to have been greatest in the egg stage but never sufficient to bring about control. One reason for this is that the

majority of eggs are laid at such low temperatures and so late in the season that they are only exposed to parasite attack for a short time, if at all, since the parasites are not as active at such low temperatures in the fall as the cankerworm adults and they do not emerge in the spring until after hatching of the host has been completed.

EFFECT ON THE TREES

Completely, or almost completely defoliated branches put out a new crop of leaves by producing new shoots about 3 weeks after defoliation. If the whole tree is stripped it becomes clothed in new foliage within a month and the untrained observer would not know it had been injured. The leaves, however, are smaller, less numerous, and a lighter green than on undefoliated trees.

The production of this second crop is evidently a drain on the food materials stored in the tree as it is associated with a striking reduction in the annual ring put on during the year of attack and also the year following. A tree which loses only about one-half of its foliage, however, generally does not put out new leaves and seems to suffer comparatively little damage to its vitality. Severely defoliated trees are always late in putting out their leaves the following spring (Figure 1).

Owing to banding and spraying, no tree has suffered complete defoliation 2 years in succession although some have lost nearly all their foliage one year and all the next. These trees show a smaller growth of leaves and on most of them dead branches have appeared (Figure 2). A few trees have died in the heavily infested woodlands in 1938 although it is not known for certain that this was due to defoliation. It has been reported from Ontario that a large number of elms have been killed by the cankerworm at one point.

Although any healthy tree will survive 2 years of complete defoliation it may be permanently injured and its vigour reduced and life shortened. It seems probable that 3 consecutive years of complete stripping would result in death.

CONTROL BY SPRAYING

The problem of spraying the city elms presented one or two difficulties. It was necessary to obtain a high percentage of control on over 1,000 trees ranging, for the most part, from 75 to 100 feet in height and nearly all overtopping or growing close to residences. Many householders feared injury to their paint. Owing to previous defoliation the trees would be slow in putting out foliage and the eggs were so numerous that many trees would be unable to grow foliage as fast as it was destroyed by the larvae. In order to reduce to a minimum the inconvenience caused by the spraying and the time and expense involved, it was very desirable to get the necessary results with one application. In the most heavily infested area where it was estimated that there would be at least 4 times as many larvae as would cause complete stripping, less than 75% control would be worse than useless as it would reduce starvation but not defoliation.

Arsenate of lead with a sticker, applied as soon as the leaves come out, has generally been recommended although Felt and Bromley (5) got fair

FIGURE 1. Two trees in spring illustrating delay in leafing out caused by defoliation previous year. Tree on right was protected by careful banding. Tree on left was stripped of leaves.

FIGURE 2. Crown of tree in spring, 1938, taken when normal trees in full leaf. Tree partially defoliated 1935 and 1936; completely stripped 1937. Note retarded growth and dead branches.

FIGURE 3. Spraying trees on side of street from top of moving sprayer.

control on apple with oil and arsenate of lead applied before the leaves separated in the bud, and Britton (4) suggests the addition of 1 U.S. pint of 40% nicotine to 100 gallons of lead arsenate spray. Francis (8) reports 90% control by spraying with an arsenate of lead and oil mixture from an autogiro as compared with 80% control from ground spraying.

As the need of an effective contact poison was indicated, preliminary tests were made in the laboratory with nicotine, derris, and pyrethrum. Eggs and elm twigs were brought into the laboratory in March and tests were made with the first 3 stages using a hand sprayer on the larvae feeding on the forced foliage. A hundred larvae were used in each test and the dead and living counted after periods of 1, 24 and 48 hours.

Pyrethrum was the only poison of the 3 which gave 100% mortality at standard strengths, and was so definitely superior that further tests were confined to determining the minimum concentration necessary with this insecticide. The pyrethrum used was in the form of a fine dust guaranteed to contain 0.8% pyrethrins. At 3 pounds to 100 Imperial gallons, 100% mortality resulted in 24 hours to all stages. At 2 pounds to 100 gallons all of the first 3 stages were killed but only 40% of the fourth. This test was repeated with the addition of arsenate of lead with similar results.

Owing to the importance of getting complete control with one application it was decided to use both arsenate and pyrethrum. In view of the danger of damage to paint on buildings, a sticker was not added. It had been found in a small experimental application in 1937 that arsenate of

FIGURE 4. Spraying outfit used.

lead adhered very well to elm foliage in spite of rain. The spray finally used was 2 pounds pyrethrum powder and 5 pounds arsenate of lead to 100 Imperial gallons of water.

The equipment purchased by the city had a capacity of 50 U.S. gallons per minute and 400 pounds pressure at the pump, and was fitted with a tank of 400 U.S. gallons (Figure 4). Two hundred feet of 1-inch hose were used nearly all the time, and a Worthley spray gun with alternative tips bored $\frac{5}{16}$, $\frac{1}{4}$, and $\frac{3}{16}$, of an inch, respectively. The sprayer was mounted on a trailer and attached to a truck in which the insecticides were carried, protected from wetting by a tarpaulin. The insecticides were weighed

beforehand into paper bags in quantities suitable for one tank filling. They were poured into the tank after the water had been started from a hydrant. No other mixing precautions were necessary.

The loss of pressure from pump to nozzle is about 33 pounds per 100 feet of hose with a $\frac{5}{16}$ inch tip. For all trees over 50 feet this tip was used and the pump was run at a pressure around 375 pounds, giving a nozzle pressure of about 300 pounds. It was found that in calm weather a height of 100 feet could be comfortably reached from the ground in this way, but a light breeze reduced the height by 10 to 15 feet, while in a moderate breeze it was difficult to reach over 65 feet. Reduction of the pressure by 50 pounds gave slightly greater height and somewhat coarser spray. To obtain sufficiently calm weather it was found necessary to commence spraying at daybreak and most of the work was done between 5 and 8 a.m. and 6 and 9 p.m.

A minimum crew of 5 men was used, including the truck driver, a foreman who ran the engine, a nozzleman, and two men to assist with the hose, filling the tank, and warning traffic. A plan showing all the trees and the order in which the streets were to be sprayed, those most heavily infested being treated first, was supplied to the foreman. Trees lining the street were sprayed first with the nozzleman standing on the slowly moving sprayer (Figure 3). The remainder were sprayed from the ground on a second trip. A great deal depends on the skill of the nozzleman who must have the knack of wetting the foliage completely without wasting spray, and of getting the necessary height by proper manipulation of the gun.

It was found possible to spray about 25 fully grown trees in an hour if they were on the roadside. On the average, however, allowing for trees in back gardens, etc., about 20 were sprayed per hour, including time for filling the tank. The average mature elm tree of 80 feet took 26 Imperial gallons of spray although some particularly large trees needed almost twice that amount.

The results of the spraying were very satisfactory in that practically complete control was obtained at a reasonable cost without serious inconvenience to the citizens. A few minutes after spraying the great majority of the larvae could be seen dropping on threads. Two samples of about 100 of these were collected from the threads and all died within an hour. Examinations of 2 trees with the aid of a fire ladder 2 days after spraying showed that the average number of larvae on 1380 leaves in the tops of the trees was 1 per 16 leaves, whereas it had been estimated that at time of spraying there were some 3 or 4 larvae per leaf. One or two branches which were accidentally missed showed complete defoliation, whereas on the remainder of the trees little or no increase in damage occurred after spraying, other than the increase in size of holes with the growing of the leaves. Finally, cocoon counts and adult counts in the fall (Tables 4 and 2) showed that the population had been reduced to a negligible quantity. From observations of the larvae it is estimated that over 90% were killed by the pyrethrum and practically all the remainder were eventually poisoned by the arsenate.

The trees sprayed on the first day were given a second application because the tops were not reached and coverage was imperfect owing to lack of experience and a moderate breeze.

Although windows near sprayed trees had to be washed, the disfigurement of paint was slight and temporary and no complaints were received. No evidence of the poisoning of birds or any other animals was obtained.

The cost was estimated at a total of not over 50 cents per average mature elm, including everything except the price of the sprayer. This could be considerably reduced in the case of less severe infestation by the elimination of the arsenate of lead. The cost of banding has been found to be about $1.75 per tree (1).

The success of the operation was due in large measure to the willingness of the city to purchase an adequate sprayer and to the city engineer in organizing an efficient crew and being ready to commence the work just as soon as the eggs had hatched.

GENERAL CONCLUSIONS

1. The fall cankerworm may be a very serious pest of elm trees in a town if the trees are numerous and the ground beneath them is not more than 60% paved.

2. Complete control by spraying is possible on trees up to 100 feet in height with a solid-stream sprayer which has a capacity of 400 pounds pressure and 50 U.S. gallons per minute. Calm weather is necessary, however.

3. Pyrethrum powder, 0.8 per cent pyrethrins, at the rate of 2 pounds per 100 Imperial gallons, will give extremely satisfactory control if applied before the larvae reach the final stage.

4. The addition of 5 pounds of arsenate of lead will ensure practically 100 per cent control if the spraying is thorough. This may be dispensed with, however, in moderate infestations. A sticker is not essential.

5. Spraying is much cheaper and more efficient than banding.

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EVALUATING ORCHARD SPRAY MATERIALS— A PROGRESS REPORT[1]

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It is axiomatic that spraying is a necessary orchard operation and it is also true that the modern pest control programme bears little resemblance to that of twenty-five years ago. It would seem to be equally true, as Anderson (1) has said, that "the problem of spray injury has been with us since the first materials were used to combat insects and diseases." Swartwout (27) expresses the same opinion: "Most spraying materials are toxic to plants as well as to the parasites against which they are employed, and the total value from spraying is reduced by the extent of damage which it causes."

REVIEW OF LITERATURE

In the literature dealing with spraying, almost all of the writers cite examples of spray injury, but generally only a slight attempt has been made to evaluate the effect of these injuries on crop production. Mills (19) as late as 1937, appears to be among the first to give definite crop values in relation to the effects of spray materials on the foliage. He states: "On the basis of bushels per acre at the planting distance of this orchard, substitution of wettable sulphur after calyx for four years increased the yield about 38 bushels per acre per year more than the straight lime sulphur schedule, while the use of wettable sulphurs for all sprays increased the yield over a hundred bushels per acre per year over the schedule of lime sulphur throughout the season." Potter's (24) data, in the same year, support the above findings, to wit: "We took as a base the yield of these same trees for three years when sprayed with lime sulphur throughout the season. The trees sprayed for another two years increased up to 109%; those sprayed with Kolofog, 125% and those with flotation sulphur increased to 132%." Petch (21), also in 1937, reports similar increases to those obtained by Mills. These findings are very significant since they were obtained in New York, New Hampshire and Quebec, by three investigators working independently of each other and using different systems for determining values. The results cited support our contention that a new method of evaluation of spray materials is needed.

Martin (16) distinguishes between what might be commonly termed the visible and hidden injuries. He states: "It is convenient when describing phytocidal activity to distinguish between acute injury, characterized by the localized killing of plant tissue (necrosis) and popularly termed "scorch" or "burn", and chronic injury which involves deep-seated physiological changes, and causes, for example, the stunting and premature drop of fruit or leaves. This distinction, though not always clear cut, is permissible because, in many cases, the two types can be ascribed to unrelated properties of the sprays or dusts responsible for the injury." The injuries recorded by experimenters have been mostly of the necrotic type. Anderson (1)

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mentions injury from lime sulphur as a burning effect on the leaves. The injury includes tip burn, marginal burn, dwarfing and "cupping" of the young leaves, and, on full grown leaves, scorch and sulphur bleach. The general effect of lime sulphur is a dwarfing of the growth regardless of whether or not direct burning is noticeable, and on the fruit a slight reduction in size and yield. Arsenicals may cause marginal or tip burn but the most common type is the "yellow leaf" condition, which is usually accentuated when lead arsenate is used in combination with lime sulphur. The older leaves, especially those around the fruit cluster, gradually turn yellow and drop. According to Merritt (18), "Applications of lead arsenate caused foliage injury, with dropping, which increased as the season advanced, apparently related to the amount applied in a given period, rather than any weather factor." This was intensified, doubtless, by lack of of rainfall. Burkholder (3), in Indiana, found, "The lime and lead spray, coming about the middle of July, caused even more serious harm. Varieties such as Grimes, Jonathan, and Ben Davis were most seriously affected, and in many cases had dropped 50 per cent of their leaves by September 1st" Wallace (28) records similar injuries to those already mentioned for lime sulphur, but he would appear to be in error when he states that "arsenate of lead is the only arsenical that has thus far proved to be safe for use with lime sulphur solution on apple trees." Under Quebec conditions, calcium arsenate is just as safe, and Sanders (25) reports: "Arsenate of lime is slightly safer than arsenate of lead with lime sulphur." Visible foliage injuries from Bordeaux mixture are cited by Sanders (25), Pike (23), and Anderson (1), as bronzing, yellowing and defoliation and the russeting of the fruit has been mentioned by nearly every experimenter.

Our experience has been that spray injury from lime sulphur has been most serious on trees weakened by severe winters, and Ginsburg (7) reports considerable arsenical injury on apple trees weakened by extreme drought.

Turning now to chronic phytocidal effects, Hoffman (11) in 1932 found that leaves sprayed with lime sulphur showed a reduction of 37.1% of their former activity. These results were not accompanied by visible injury, such as tip or marginal burning. The treated leaves appeared perfectly normal except for the slight deposit left by the spray after drying. He states that "any reduction in leaf efficiency early in the season may have a marked influence on such characteristics as setting of fruit, biennial bearing, etc." In 1933, Hoffman (12) records lime sulphur as decreasing the value of leaves by 16.4 and 31.3%. In 1934, Overholser and Overley (20) state: "The fruit from the former trees (those sprayed with calcium arsenate with "safeners") was of better colour and size than from those receiving lead arsenate, indicating greater photosynthetic capacity of leaves receiving calcium arsenate with "safeners". This advantage may be due to the presence of calcium, because according to Clore (5) "the assimilation averages of treated and untreated leaves correspond quite closely with the exception of calcium sprayed leaves in which CO^2 intake was markedly increased." Spuler (26) also deals with sizing of the fruit and says: "A reduction in the size of the fruit will occur if the leaf area is reduced at any time during the growing season." Heinecke (9), in 1934, points out the importance of photosynthesis in relation to annual bearing, through the activity of leaves and roots, and, in 1936, he (10) deals with

some fungicides which apparently have no influence on photosynthesis. Whetzel (29) describes the effect of lime sulphur injury as follows: "At Cornell University, investigators have found that lime sulphur affects the apple leaf in such a way that 40 to 60 per cent of its efficiency in manufacturing sugar is lost. Sugar, which is the principal substance manufactured by leaves, is used in the growth of the tree and in the formation of next year's fruit buds." Hoffman (13) says that, "Lime sulphur injury to apple leaves is largely due to a decrease in the rate of photosynthesis rather than an increase in the rate of respiration."

A special case of phytocidal action arises when sulphur-containing fungicides are applied during the blossoming period. MacDaniels and Furr (17) have shown that sulphur dusts deposited on the stigmas of apple blossoms inhibit pollen germination and hence reduce the setting of the fruit. Hamilton (8) minimizes this factor and says that the practice of spraying during full bloom for apple scab control is feasible from a commercial point of view. However, data obtained by Petch (21) showed a crop reduction of over 22% in McIntosh for the 4-year period 1934 to 1937 following an application of bentonite sulphur 3 lbs. and hydrated lime 3 lbs. to 40 gals. In each of the years there was a reduction amounting in 1936 to over 35%. It has been suggested that the injury may have been due to the hydrated lime and not the sulphur, and this point will be taken up in future experiments.

As stated previously, there has been very little interest in these invisible phases of the problem, or such would seem to be the logical conclusion from a review of the literature. The bases of value seem to have been limited to the effect on apple scab *(Venturia inaequalis)* and a few insects, with references to the codling moth *(Carpocapsa pomonella)* far outnumbering all the others. In certain situations these pests may be deserving of the attention they have received, but in many large areas it would seem they have occupied a position out of proportion to their economic importance in relation to the spraying problem as a whole. It is admitted that in those sections where these pests are very serious, the advantages in growth from changes in the spray programme might have to be largely sacrificed for the sake of pest control. Dye (6) summarized the situation as follows: "In general, the exact amount of disease present on the fruit at harvest time is regarded by investigators as representing the comparative efficiency of the different fungicides and insecticides and statistical methods are applied to that data in judging the efficiency of the materials. If observations on visible differences are included they have relatively little weight. Actually, if statistical methods could give us a formula taking into account growth differences, the value of some materials might quite conceivably be represented by a decided minus amount of infection as compared with that developing with the standard treatment even though the actual percentage of disease present on the fruit at harvest time was greater." We have tried to answer partially the need for such a system of evaluation and our method has been applied in a series of experiments starting in 1934. The points considered were as follows: apple scab, russeting, end-burn, the principal insect pests, windfalls, the number of apples, weight of the harvested crop, trunk girth, terminal growth, bud size and ash content of the foliage. These criteria

would not meet the standards for growth measurements as set forth by Blake and Davidson (2), yet our results have been so consistent that we might argue they were satisfactory for our purposes. At least, they were the most obvious points suited to our limited knowledge and time.

MATERIALS AND METHODS

In planning a series of comparative experiments of sprays, the selected trees should be as nearly similar as possible, so that any differences in growth or production would be more significant. In our experiments the selected trees were the variety McIntosh, because, in addition to being our principal commercial variety, it is very susceptible to apple scab and most of our major apple pests. The trees were started as 2-year old nursery stock and had been planted for 15 years when the experiments were begun. The culture was the sod mulch system, plus annual applications of a complete fertilizer (9-5-7), at a rate of one-half pound per year of age, applied at the pink bud stage. The plots were in a tile drained orchard and on a western slope, which made the water and air drainage of the plots comparable. The spraying was done by the same workmen and the same sprayer, so as to obtain as closely as possible a similar degree of efficiency of application. In order to avoid appreciable differences due to weather conditions, the plots received the individual applications on the same day. No attempt was made to use an extra large number of applications in order to attain perfect control of pests or to accentuate growth differences. The plan followed was to apply the number of sprays recommended by the spray service, so that any beneficial results, which might be obtained, would be applicable to commercial orcharding and economically sound. The trees selected for production and growth records within each plot were

Table 1.—Spray schedules

Plot	Applications*	Spray mixtures
1	Nos. 1, 2, 4, 5,	Lime sulphur 1–40; Calcium arsenate 1¼–40
2	Nos. 1, 2, 4, 5 No. 3	Lime sulphur 1–40; Calcium arsenate 1¼–40 Bentonite sulphur 3–40; Hydrated lime 3–40; applied during full bloom.
3	Nos. 1 and 2 Nos. 4 and 5	Lime sulphur 1–40; Calcium arsenate 1¼–40 Bentonite sulphur 3–40; Hydrated lime 3–40; Calcium arsenate 1¼–40
4	No. 1 Nos. 2, 4 and 5	Lime sulphur 1–40; Calcium arsenate 1¼–40 Lime sulphur 1–40; Calcium arsenate 1¼–40 Iron sulphate 4–40.
5	Nos. 1 and 2 Nos. 4 and 5	Lime sulphur 1–40; Calcium arsenate 1¼–40 Lime sulphur 1–40; Calcium arsenate 1¼–40 Iron sulphate 4–40.
6	Nos. 1 and 2 Nos. 4 and 5	Lime sulphur 1–40; Calcium arsenate 1¼–40 Flotation sulphur cream 5–40; Calcium arsenate 1¼–40

Applications:— (1) green tip; (2) pink bud; (3) blossom; (4) calyx; (5) first cover.

* In addition to the above and for the control of apple maggot, all plots received two cover sprays of an arsenical alone, except in 1937 when the arsenical was combined with fungicides.

chosen at random but each had a trunk circumference of 21 inches at a height of 1 foot above ground level. Four such trees were used out of the 50 trees in each block.

The spray schedules outlined in Table 1 were used every year except in 1937, when plots 1, 2, 4, 5, and 6 received six, and plot 3 seven post blossom applications of fungicides. As the experiment with flotation sulphur cream (ferrox type) was first introduced in 1937, the results from it are not comparable with those obtained from other mixtures. The combination, lime sulphur and calcium arsenate, was used as a standard of comparison.

RESULTS

Disease and Pest Control

TABLE 2.—CONTROL OF APPLE SCAB

Plot	1934			1935		
	No. apples	No. scabby	Scabby %	No. apples	No. scabby	Scabby, %
1	3502	0	0	5282	12	.227
2	3614	5	.138	3912	2	.051
3	3840	6	.156	8122	224	2.819
4	4236	4	.094	4686	45	.566
5	4309	2	.046	6860	54	.918
	1936			1937		
1	827	228	27.57	6576	587	8.93
2	526	82	15.59	4544	895	19.70
3	933	408	43.73	7037	5314	75.52
4	665	68	10.23	4480	2392	53.17
5	633	181	28.59	6203	2146	34.60
6				7823	3240	41.42

1934–1937

Plot	No. apples	No. scabby	Scabby, %
1	16187	827	5.11
2	12596	984	7.81
3	19932	5952	29.86
4	14067	2499	17.76
5	18005	2383	13.23

NOTES:

(a) Severe frost on May 15, 1936, killed most of the blossoms.

(b) By 1937, two trees in Plot 4 showed very serious effects of winter injury, which occurred in 1933–34.

The data in Table 2 show that both lime sulphur plots had less scab for the 4-year period and markedly less in 1937, a year of serious scab prevalence, than the other combinations. Iron sulphate added to lime sulphur at the rate of 4 pounds to 1 gallon of lime sulphur resulted in an average increase of scab over the lime sulphur plots of 8.11% when 2

applications were made, and 12.65% with 3 applications. Burrell (4) noted that in the above proportions, "It almost completely eliminated the effectiveness of the mixture in 'burning-out' scab lesions already visible on the foliage." Kelsall (14) shows its use results in more scab. Bentonite sulphur gave poorer control of apple scab in each of the 4 years and, in 1937, it offered little protection to the fruit. Flotation sulphur cream was superior in scab control to bentonite sulphur in 1937.

Kelsall and Hockey (15) graded scab infections as follows: "A" those apples which show a small speck; "B" those apples showing a moderate scabbed area; and "C" apples so seriously scabbed as to be definitely culls. Our data would probably have been of more value had we followed some such classification. In making our examinations, the apples were considered to be scabby regardless of the size of the infections. It might be well to mention that our data were not taken on the picked fruit until it had been in storage more than a month, which would allow storage scab to start. We consider that some such delay gives apple scab a chance to develop in storage and more nearly approximates the actual condition of the fruit at the time it is sold than does an examination at picking time. Possibly, a better value of fungicidal efficiency would be obtained if examinations were made over longer periods.

TABLE 3.—RUSSETING AND END-BURN INJURY

Plot	1934			1935		
	No. apples	Russet	End-burn	No. apples	Russet	End-burn
1	3502	110	0	5282	531	196
2	3614	121	0	3912	469	416
3	3840	224	0	8122	422	284
4	4236	333	0	4686	264	0
5	4309	118	0	6860	542	0
	1936			1937		
1	827	53	0	6576	360	0
2	526	22	0	4544	190	0
3	933	30	0	7037	70	0
4	665	49	0	4480	107	0
5	633	49	0	6203	163	0
6				*7823	126	0

1934–1937

	No. apples	Russet	End-burn	Russet, %	End-burn, %
1	16187	1054	196	6.51	1.21
2	12596	802	416	6.37	3.30
3	19932	746	284	3.74	1.43
4	14067	753	0	5.35	.0
5	18005	872	0	4.84	.0
6	7823	126	0	1.61	.0

* Plot 6. Records for 1937 only.

The common injuries to the fruit by sprays are burning around calyx, which we have termed "end-burn", and russeting. In 1935, there was a considerable amount of "end-burn", but the addition of iron sulphate prevented this type of injury. Russeting on the average was less from bentonite sulphur than from the other combinations. Since these injuries amount to as much as 10% annually with some combinations, it is evident that they should not be overlooked, because, in years like 1934, they result in more lower grade apples than does apple scab.

TABLE 4.—INSECT INJURIES 1934–1937

Plot	Codling moth	Plum curculio	Apple curculio	Bud-moth	Green fruit worms	Lesser Apple worm	Total injuries	Percent injured
1	140	28	34	61	58	13	334	2.06
2	99	41	29	83	58	8	318	2.52
3	110	59	25	117	146	21	478	2.39
4	157	48	31	105	69	5	415	2.95
5	119	79	59	85	104	0	446	2.48
6*	0	0	0	5	1	0	6	0.08

* Plot 6—Records for 1937 only.

In Quebec, calcium arsenate is used almost entirely as an insecticide for apples, therefore, it was the only one used in these experiments. (In 1938, nicotine-bentonite was employed in all the sprays in one experiment in an effort to lessen the injuries from arsenic and also to avoid arsenical residues. According to Martin (16) no record can be found of any damage caused, in the use of nicotine washes and dusts, by nicotine itself.)

The injuries were recorded from the apples which dropped or were harvested commencing with the "June crop". The differences between any of the plots is not significant, and the data indicate that calcium arsenate was a satisfactory insecticide in the combinations used.

TABLE 5.—TOTAL INJURIES TO APPLES 1934–1937

Plot	No. apples	Scab	Russet	End-burn	Insects	Total	Per cent injured	No. clean apples
1	16187	827	1054	196	334	2411	14.89	13776
2	12596	984	802	416	318	2520	20.01	10076
3	19932	5952	746	284	478	7460	37.43	12472
4	14067	2499	753	0	415	3667	26.07	10400
5	18005	2383	872	0	446	3701	20.55	14304
6*	7823	3240	126	0	6	3372	43.10	4451

* Plot 6—Records for 1937 only.

Since any blemish on the fruit reduces its grade, all the injuries noted in our experiments have been assembled in Table 5, rather than base the values of the combinations on apple scab only. A study of the results

indicates that the lime sulphur and calcium arsenate combination gave the best quality fruit; the same combination plus iron sulphate is in second place, and the bentonite sulphur plus calcium arsenate combination in third place. However, if the effects of some materials on the foliage result in increased crops, as would appear to be the case in the bentonite sulphur-calcium arsenate plot, and if all the defective apples were discarded, the position of the plots would change from 1, 2, 5, 4, 3 to 5, 1, 3, 4, 2.

However, the number of apples might not be the final answer to the problem, because there might be a difference in the size of the fruit or in the percentage of "drops". In order to evaluate these possibilities, the harvested crop was counted and the fruit was weighed. The data for these points are contained in Table 6.

TABLE 6.—NUMBER AND WEIGHT OF APPLES 1935–37

Plot	1935		1936		1937		Total apples	Total weight	Average weight in ozs.
	No. apples	Weight in lbs.	No. apples	Weight in lbs.	No. apples	Weight in lbs.			
1	4321	1069	753	186	5437	1132	10,511	2387	4.20
2	3277	803	377	88	3631	787	7,285	1678	3.70
3	6651	1630	806	189	5616	925	13,073	2744	3.36
4	3806	1018	573	157	3308	654	7,687	1829	3.81
5	5120	1343	497	128	4995	1091	10,612	2562	3.86
6*					6066	1197			2.99

* Plot 6—Records for 1937 only.

The results show that in respect to the average size of the apples the plots ranked—from high to low—1, 5, 4, 2, 3, but in total weight of fruit, 3, 5, 1, 4, 2. Bentonite sulphur sprayed trees produced the largest crops, but the apples were larger on trees treated with lime sulphur. None of the apples harvested were below the minimum marketable size for the variety, namely $2\frac{1}{4}$ inches in diameter.

TABLE 7.—NUMBER AND PERCENTAGE OF "DROPS" 1934–1937

Plot	1934	1935	1936	1937	Total no. apples	Total no. "drops"	"Drops", %
1	604	961	74	1139	16,187	2778	17.16
2	899	637	139	913	12,596	2588	20.55
3	752	1471	127	1421	19,932	3771	18.92
4	625	860	92	1172	14,067	2749	19.58
5	630	1640	136	1208	18,005	3614	20.07

In Table 7 the "drops" include all the apples that fell from shortly after petal-fall to just before harvest. The differences in percentages of "drops" are not sufficiently large to be significant.

Effect on Tree Growth and Development

The second phase of a study of spray materials should deal with the possible effects they may have on tree growth and development. This phase would appear to be very important, because the crop, which after all

is the ultimate objective in apple culture, should be relative to the development of the trees. We have tried to correlate the amount of crop with development of trunk girth, terminal growth, bud length and the ash content of the leaves.

TABLE 8.—TRUNK GIRTH OF TREES 1934–1937

Plot	Average girth of trunk in 1934	Average girth of trunk in 1937	Increase in trunk girth
1	21 inches	26.4 inches	5.4 inches
2	21 inches	25.0 inches	4.0 inches
3	21 inches	25.9 inches	4.9 inches
4	21 inches	24.5 inches	3.5 inches
5	21 inches	27.0 inches	6.0 inches

When the experiments were started, each of the trees had a trunk girth of 21 inches at a height of one foot above the surface of the soil. The trunk growth of the trees from May 1934 to September 1937 is given in the above table. Although lime sulphur (Plot 1) and lime sulphur-iron sulphate (Plot 5) had greater trunk girth than bentonite sulphur (Plot 3), they did not have as many apples or as great a weight of fruit.

TABLE 9.—TERMINAL GROWTH OF 100 TWIGS IN INCHES

Plot	1934	1935	1936	Total
1	328 $\frac{3}{4}$	465	728 $\frac{1}{2}$	1522 $\frac{1}{4}$
2	339 $\frac{1}{2}$	305	711 $\frac{3}{4}$	1356 $\frac{1}{4}$
3	383	532	686	1601
4	258 $\frac{1}{4}$	431	611	1300 $\frac{1}{4}$
5	255 $\frac{1}{4}$	440	759 $\frac{3}{4}$	1455

The terminal growth was determined from the measurement of 25 twigs annually on each of 4 trees. The twigs were taken at random from the bearing branches on the lower portion of the trees. In the lime sulphur and lime sulphur-iron sulphate plots, there was less terminal growth than in the bentonite sulphur plot.

TABLE 10.—MEASUREMENTS OF 100 FRUIT BUDS IN 16THS INCHES

Plot	1934	1935	1936	Total
1	481	605	616	1702
2	468	586	636	1690
3	521	614	640	1775
4	525	561	622	1708
5	542	579	612	1733

The development of the buds was determined from the measurement of the length of 25 buds annually on each of the 4 trees, selected at random from the 2-year wood on the lower portion of the trees. In the lime sulphur and lime sulphur-iron sulphate plots the buds were shorter than

in the bentonite sulphur plot. In 1928 Petch (22) found that buds on trees sprayed with lime sulphur were much smaller than those receiving bentonite sulphur, the average length of buds being respectively 0.40 inch and 0.49 inch.

TABLE 11.—PERCENTAGE OF ASH IN 5 GRAMS OF DRIED LEAVES

Plot	1934	1935	1936	Average
1	8.43	6.34	8.40	7.72
2	8.73	6.37	6.4	7.17
3	11.54	6.69	6.8	8.34
4	9.14	6.74	7.0	7.62
5	10.94	6.86	8.3	8.70

In order to establish a relationship between the foliage and the amount of crop, the mature leaves from 100 spurs were gathered in each experiment for the years 1934 to 1936 inclusive. The leaves were chemically treated to remove the spray residues, dried in a desiccator, and 5 grams were reduced to ash. When the total ash content for the 3-year period is compared to the crop for the same period there seems to be a fairly close relationship between the ash content of the leaves and the weight of apples produced.

REMARKS

The data presented indicate that an increase in crop production may be correlated with an increase in bud size, twig growth and the ash content of the leaves, and that the differences in these may be due to different fungicides used.

The object in spraying should be to employ materials which will protect the tree and the fruit against insects and plant diseases without phytocidal action. In the past, in nearly every instance, the condition of the fruit at picking time has been used as the basis for determining the value of spraying materials. It is believed that the best criterion for determining the value of insecticides and fungicides is the weight of marketable and clean fruit.

The data presented in relation to our experiments, as well as the supporting evidence, show that there should be an evaluation of spray materials in relation to tree growth and foliage development and total crop production in addition to the protection given to the fruit.

The various phases considered in our experiments may have been insufficient, or should have been analyzed differently, but, at least, an attempt has been made to evaluate some phases of the spraying problem, which hitherto have received very little attention.

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FACTORS INFLUENCING THE REDUCTION OF METHYLENE BLUE IN MILK[1]

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During the past few years, several modifications in the technique of the methylene blue reduction test have been proposed. The value of preliminary incubation at a lower temperature (16, 26, 36), of periodical inversion of the tubes to eliminate the errors arising from the creaming effect (10, 15, 16, 29, 36), of protection against light (8, 9, 35), and of an improved salt of methylene blue (31, 32), have all been stressed, and several of these modifications have been incorporated into official methods (5, 20). It seemed opportune, therefore, to study in some detail the influence of various factors upon the efficiency of this test.

The value of periodical inversion of the tubes to re-incorporate the cream and accompanying bacteria and body cells has been pointed out by various workers (10, 15, 16, 22, 26, 29, 36), and mixing at 30 minute intervals has been officially adopted in England (20). Its adoption on this Continent has been opposed (33, 34) on the grounds that the accuracy of the test is not significantly increased thereby, while the technique is complicated unduly. These objections have been dealt with in another paper (16) and need not be reiterated here. Admittedly, inspection and inversion of the tubes at 30 minute intervals proves inconvenient, especially in the smaller plants. If less frequent mixing yields satisfactory results, objections to this modification on these grounds would lose much of their weight.

The fact that maximum plate counts occur in the vicinity of 30 to 32° C. with 48 hour incubation (21, 37) suggests the possibility that the optimum temperature for incubation in the methylene blue reduction test may also be lower than that at present used (37° C.). The literature records few attempts to determine the optimum temperature except within fairly wide limits, while there are certain differences of opinion recorded. As originally introduced (4) a temperature of 38 to 39° C. was employed. Arup (3), however, recommended 28 to 29° C. since samples refrigerated for 24 hours reduced more rapidly at this than at higher temperatures. Rahn (23), on the basis of pure culture studies, reported 38 to 40° C., as the optimum. Hastings, Davenport and Wright (12) compared temperatures of 28, 33 and 38° C., finding the reduction time to shorten as the temperature was raised. A temperature of 37° C. was officially adopted in 1923 on this Continent (2). In 1934 the present author found the average reduction time of 17 samples of raw milk was 27% longer at 32° C. than at 37° C. Wilson (36), comparing temperatures of 35, 37 and 39° C. reported that with a poor grade of raw milk (average reduction time 82 minutes) the reduction time increased with increase in temperature; with Grade A raw and pasteurized milks, however, the reverse held true.

An improved form of dye (methylene blue thiocyanate) has now been adopted (5, 6) as a result of the studies of Thornton *et al.* (31, 32). In

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addition, the concentration of dye in milk has been increased from approximately 1 : 700,000 to 1 : 300,000 (5, 6). Although the change in the form of dye does not affect the reduction time, the increase in concentration may be expected to delay reduction (8, 12). Thornton and Sandin (31) report an increase of approximately 30 minutes for all classes of milks[3].

While collaborating in the testing out of methylene blue thiocyanate in 1934, some anomalous results were encountered. Where hourly mixing of the tubes was employed, marked disparities between reduction times for different dye concentrations sometimes appeared, reduction being significantly slower with the stronger dye concentrations. Where tubes were incubated undisturbed, no discrepancies of similar magnitude were encountered. Since an even stronger dye concentration is now being recommended, it appeared desirable to determine more definitely the possible influence of mixing upon reduction time with the old and new dye concentrations.

In addition to the error resulting from undisturbed creaming, the standard reduction test suffers from the disadvantages that (1) in promptly cooled milk the bacteriostatic activity may delay bacterial growth (14, 17) while (2) the temperature of incubation is not favourable to the growth of many species commonly concerned in the spoilage of milk. To avoid these objections, the author in 1930 (15) recommended a preliminary incubation at 12.8° C. for 18 hours. With this modification, reduction time at 37° C. was generally greatly shortened and keeping quality was indicated with greater accuracy than with any of the other tests employed (plate count, direct microscopic count, standard reduction test or rate of acidity increase). Furthermore, incubation at 37° C. could be started early the following day, and concluded within ordinary working hours.

The shortcomings of the reduction test when applied to fresh milk were also pointed out in 1931 by Skar (26), who advocated holding samples at 13 to 16° C. for 24 hours before starting the test. With this modification, coupled with the mixing of the contents of the tubes at frequent intervals, reduction times showed a much more satisfactory agreement with the keeping quality.

The employment of the reduction time of tubes incubated at 15.5° C. in place of organoleptic tests for keeping quality was suggested by Hiscox *et al.* in 1932 (13). Further studies by Provan *et al.* (22) indicated the necessity for redispersing the bacteria twice daily. Where this was done, the agreement between reduction time and keeping time was very close.

In 1935 Wilson (36) advocated that producers' samples be held at atmospheric (outdoor) temperature for a definite period before being analysed. In this way, differences between lightly and heavily contaminated milks are made more evident, since the bacteriostatic action is less pronounced in the latter. Reports have come from Scotland (18) and Wales (28) that this procedure permits a large percentage of high count winter milks to meet the standard of 5½ hours reduction time (20). This apparently is not the case in England (19). Evidently milk heavily contaminated through faulty production methods may escape detection when promptly cooled and held at low temperatures. While the principle under-

[3] Since these studies were commenced, Frayer (10) has reported increases of the order of 100% on all grades of milk.

lying Wilson's proposal is sound, it would seem preferable to maintain a definite temperature for preliminary incubation, thus avoiding the objections mentioned above and permitting direct comparison of reduction times from one season to another. This view appears to be receiving increasing support from British bacteriologists (7, 19)[4].

Part I. Influence of Temperature of Incubation and Frequency of Mixing upon Reduction Time of Fresh and Refrigerated Samples

Since the oxygen content of the milk is affected by both temperature and frequency of mixing the contents of the tubes, concurrent studies were made of the influence of these two factors upon reduction time.

In view of the findings reported by Wilson (36) it seemed probable that the optimum temperature for the ordinary run of market milks might lie somewhat below body temperature. Accordingly, temperatures of 32.5°, 35°, and 37.5° C. were first selected for comparison.

Tests in 1930 (15) had failed to bring out any marked differences between mixing at intervals of $\frac{1}{2}$, 1 and 3 hours. In the first series of the present studies, mixing after 1, 2 and 3 hours was compared with no mixing, but in view of the known variability of reduction times on unmixed high grade milks, the unmixed set was discontinued and $\frac{1}{2}$ hour mixing substituted in subsequent series.

METHODS

Samples of mixed night's and morning's milk were taken from the weigh-can at 3 local plants. Methylene blue thiocyanate was added to give a concentration of 1 : 300,000; 10 ml. portions were measured into sterile stoppered test tubes and incubated at once in thermostatically controlled water baths with covers. Mixing consisted of a single inversion of the tube at the appropriate hour; tubes showing definite signs of decolorization were left undisturbed until reduction was completed. Tubes were examined every 15 minutes and were considered reduced when the colour agreed with that of a control tube containing no dye.

The remaining portions of the samples were held at approximately 5° C. for 24 hours, when a second set of samples was prepared and handled in an identical manner. The purpose here was to note any possible lowering of the optimum temperature of incubation as a result of refrigerated storage as reported by Arup (3).

RESULTS

Data obtained from an examination of 26 samples, both fresh and after 24-hour refrigeration, are presented in Table 1. To facilitate comparison, the average reduction time for tubes of fresh milk incubated at 37.5° C. and mixed hourly has been given a value of 1.0, and all other values are expressed as multiples. In both the first and third series, where the average reduction time for the series of fresh samples was approximately 6 hours,

[4] Since this paper was prepared, similar views have been expressed by Thomas and Hobson (Welsh J. Agr. 14 : 247-60. 1938) and by Chalmers (J. Dairy Research, 9 : 351. 1938).

TABLE 1.—RELATIVE REDUCTION RATES AT 32.5°, 35°, AND 37.5° C. WITH VARIOUS FREQUENCIES OF MIXING CONTENTS OF TUBES

—	Incubation temperature	32.5° C.					35° C.					37.5° C.				
	Frequency of mixing (hrs.)	0	½	1	2	3	0	½	1	2	3	0	½	1	2	3
A. Fresh	Series I	1.30	—	1.18	1.20	1.20	1.23	—	1.09	1.10	1.13	1.26	—	1.00	1.03	1.03
	Series II	—	0.92	0.92	1.00	1.01	—	0.92	0.92	1.00	1.00	—	1.00	1.00	1.04	1.12
	Series III	—	1.22	1.23	1.33	1.40	—	1.02	1.09	1.17	1.21	—	1.02	1.00	1.04	1.07
B. Held 24 hrs. at 5° C.	Series I	1.27	—	0.92	0.96	1.01	1.15	—	0.85	0.91	0.93	1.18	—	0.89	0.90	0.95
	Series II	—	0.64	0.64	0.72	0.77	—	0.62	0.62	0.63	0.64	—	0.65	0.64	0.67	0.68
	Series III	—	0.96	0.97	1.06	1.15	—	0.86	0.89	0.95	0.96	—	0.84	0.82	0.84	0.92

Average reduction time for *fresh* samples of each series at 37.5° C. with *hourly* mixing is given a value of 1.0; reduction times for all others are shown as multiples of this.

the reduction time was shortest at 37.5° C. increasing as the temperature was lowered. The refrigerated portions, on the other hand, showed a tendency to reduce slightly faster at 35° C. while the reduction times for the three temperatures showed less spread than with the fresh samples. The milks in the second series were of poorer quality, averaging less than 3 hours; these tended to reduce more rapidly at the lower temperatures both while fresh and after 24 hours refrigeration. This is in agreement with Wilson's (36) findings. The refrigerated samples of this series show a much more marked shortening of reduction time than in the case of the better grades in Series I and III. In all three series reduction time was generally shortened by holding for 24 hours at 5° C. before conducting the test. The reason for the lack of agreement between these results and the findings of Arup (3), Frayer (9) and Wilson (36) probably lies in the greater age of these milks when sampled, with a corresponding weakening of the growth inhibiting factor (17).

With respect to frequency of mixing, a very close agreement was noted between the results from ½ and 1 hour mixings, the average for 156 tubes being only 0.6% greater for the longer interval. (In a further series of tests on 100 samples, reduction time averaged 0.86% longer for hourly mixing.) As the interval between mixings is increased beyond one hour the reduction time tends to increase to a somewhat greater extent, particularly at temperatures below 37.5° C. At 37.5° C. the difference between 1- and 2-hour mixings is so small that it seems doubtful whether, in ordinary routine testing, mixing more frequently than every 2 hours would be justified (see data on individual samples in Table 2). However, for the majority of the present studies, inversion of tubes at hourly intervals was adopted as standard practice.

Although the number of samples in Series I was not large, the shortening of reduction time through periodical inversion of tubes is quite marked. This effect appears to be greatest at 37.5° C.

Part II. Influence of Temperature of Incubation, Mixing and Dye Concentration upon Reduction Time with and without Preliminary Incubation

Since the foregoing studies indicated that the optimum temperature was rarely lower than 35° C., temperatures of 35°, 37.5°, and 40° C. were adopted for subsequent studies. In place of the retesting of refrigerated portions, 10 ml. portions were subjected to preliminary incubation at 12.8° C. for 18 hours before being incubated at the higher temperatures (15).

Sets of tubes were prepared containing methylene blue thiocyanate in concentrations of 1 : 300,000 and 1 : 700,000 to determine the influence of the higher dye concentration upon reduction time, both with and without mixing.

During incubation tubes were inverted hourly, although for comparison a set of unmixed tubes was included with both dye concentrations at 37.5° C.

The samples examined comprised combined nights' and mornings' milk delivered to a local plant. Ten-ml. portions were pipetted into sterile

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TABLE 2.—INFLUENCE OF FREQUENCY OF MIXING UPON REDUCTION TIME AT 37.5° C. (1 : 300,000 METHYLENE BLUE THIOCYANATE)

Sample No.	Average of triplicate tubes					
	Not mixed		Mixed every hour		Mixed every 2 hours	
	Average reduction time (minutes)	Maximum variation (minutes)	Average reduction time (minutes)	Maximum variation (minutes)	Average reduction time (minutes)	Maximum variation (minutes)
1	660	30	465	0	480	0
2	735	45	315	0	345	0
3	830	45	415	30	440	15
4	645	75	390	0	400	15
5	330	0	270	0	270	0
6	530	30	465	0	455	30
Average for 6 samples	622		387		398	

Sample No.	Reduction times of single tubes			
	Fresh		Refrigerated 24 hours at 5° C.	
	Mixed every hour	Mixed every 2 hours	Mixed every hour	Mixed every 2 hours
7	465	465	375	375
8	415	415	335	345
9	380	380	300	315
10	435	465	405	405
11	435	475	405	405
12	460	465	405	405
13	170	195	125	120
14	200	200	90	90
15	140	170	150	150
16	185	180	150	165
17	300	300	225	225
18	270	270	180	180
19	—	—	115	120
20	—	—	240	240
21	—	—	330	330
22	430	465	355	380
23	410	420	330	325
24	275	290	210	250
25	415	415	340	335
26	300	300	245	245
27	385	395	240	225
28	165	180	190	195
29	245	255	210	220
30	405	405	345	345
Average	328 min.	338 min.	262 min.	266 min.

stoppered test-tubes, the dye solution added, and after a single inversion, placed in the water bath. In the last 3 series, it was necessary to hold samples overnight at 5° C. before running the tests. Comparative tests showed that this practice had little or no effect upon reduction time.

RESULTS

A. Influence of Incubation Temperature

In order that the trend in relationship between milk quality and optimum incubation temperature might be more evident, samples have been grouped into classes on the basis of their reduction time at 37.5° C. with hourly mixing. In Table 3 are presented data on samples not subjected to preliminary incubation at 12.8° C., while in Table 4 are found

TABLE 3.—INFLUENCE OF INCUBATION TEMPERATURE UPON RATE OF REDUCTION OF METHYLENE BLUE IN MILK. TUBES INVERTED EVERY HOUR. NO PRELIMINARY INCUBATION AT 12.8° C.

Dye concentration	1 : 300,000				1 : 700,000			
Incubation temperature		35°	37.5°	40°		35°	37.5°	40°
Samples reduced in	No. of samples	min.	min.	min.	No. of samples	min.	min.	min.
0 – 1 hour	3	30	35	*15*	8	23	*17*	21
1 – 2 hours	6	*72*	75	103	6	*93*	100	95
2 – 3 hours	5	170	*160*	166	4	181	*170*	193
3 – 4 hours	9	*232*	*221*	231	6	227	*226*	236
4 – 5 hours	20	322	*281*	289	16	303	282	*280*
5 – 6 hours	19	364	*342*	343	17	364	339	*330*
6 – 7 hours	16	441	404	*402*	11	427	*395*	402
7 – 8 hours	10	476	455	*443*	7	503	*466*	491
8 – 9 hours	4	539	519	*491*	1	560	525	*510*
9 – 10 hours	5	592	581	*565*	0	–	–	–
>10 hours	1	810	690	*685*	1	700	625	*585*
Averages		352	327	328		303	283	285

TABLE 4.—INFLUENCE OF INCUBATION TEMPERATURE UPON RATE OF REDUCTION OF METHYLENE BLUE IN MILK. TUBES INVERTED EVERY HOUR. PRELIMINARY INCUBATION AT 12.8° C. FOR 18 HOURS

Dye concentration	1 : 300,000				1 : 700,000			
Incubation temperature		35°	37.5°	40°		35°	37.5°	40°
Samples reduced in	No. of samples				No. of samples			
0 – 1 hour	10	41	36	*35*	10	*43*	*43*	*43*
1 – 2 hours	19	*93*	94	95	12	112	*110*	116
2 – 3 hours	17	*154*	*154*	163	10	*135*	147	157
3 – 4 hours	12	*217*	220	230	4	*198*	224	220
4 – 5 hours	10	*265*	284	293	1	330	*300*	360
5 – 6 hours	4	*341*	344	353	1	375	*360*	*360*
6 – 7 hours	7	436	*416*	431	7	406	388	*374*
7 – 8 hours	1	*465*	*465*	525	1	450	450	*420*
8 – 9 hours	4	546	*526*	559	1	555	*500*	540
Averages		204	204	212		180	179	182

similar data for samples given the preliminary incubation. Since the two dye concentrations reveal differences of much the same order in both tables, the data have been combined to obtain the values shown in Figures 1 and 2.

It will be observed in Figure 1 that up to the fourth hour there is little difference in average reduction time at the 3 temperatures; beyond

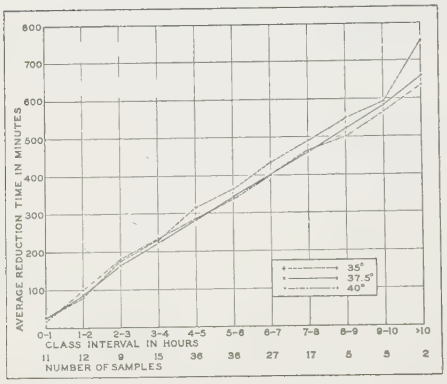

FIGURE 1. Relative reduction times at 35°, 37.5°, and 40° C. No preliminary incubation.

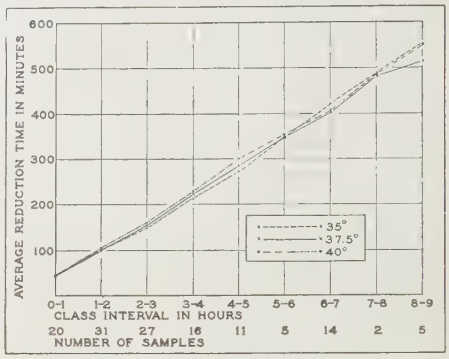

FIGURE 2. Relative reduction times at 35°, 37.5°, and 40° C. with preliminary incubation.

this point, reduction is definitely slower at 35° C. With milks lasting over 8 hours, reduction is somewhat faster at 40° C. than at 37.5° C. Figure 2 indicates that, contrary to expectation, preliminary incubation at 12.8° C. for 18 hours has very little effect upon the optimum temperature for subsequent incubation. Although reduction is slightly faster at 35° C. up to the 6th hour, the results at the three temperatures agree closely. The optimum incubation temperature increases as the proportion of saprophytic contaminants decreases. As milk supplies improve, therefore, the optimum temperature will increase. There appears to be no good reason for changing the present incubation temperature of 37° C. (2).

B. Influence of Dye Concentration

Increasing the dye concentration to 1 : 300,000 results in a definite increase in reduction time, although since a certain number of samples run at 1 : 300,000 concentration were not run at the lower concentration a direct comparison of the data in Tables 3 and 4 cannot fairly be made. In Table 5, however, are summarized data on 109 samples in which a direct comparison is possible. (Individual data for 97 of these samples have been plotted in Figure 3). Where the tubes are mixed every hour, it will be seen that with the higher dye concentration the average reduction time is 18% longer where no preliminary incubation is employed; with preliminary incubation the average reduction time is 36% longer. Values of much the same order are obtained where the comparison is made on tubes not inverted every hour.

It will be observed that the higher dye concentration delays reduction to a greater degree following preliminary incubation at 12.8° C. This may be due to the greater sensitiveness of actively growing cells to unfavourable

TABLE 5.—REDUCTION TIMES OF 109 MILK SAMPLES SUBJECTED TO VARIOUS MODIFICATIONS OF THE METHYLENE BLUE TEST. INCUBATION AT 37.5° C.

Preliminary incubation	None				18 hours at 12.8° C.			
Dye concentration[1]	1 : 300,000		1 : 700,000		1 : 300,000		1 : 700,000	
Mixing frequency	1 hour	0	1 hour	0[2]	1 hour	0	1 hour	0
Average reduction time (minutes)	386	552	329	479	202	271	148	197
Relative reduction times	0.81	1.16	0.69	1.0	0.42	0.57	0.31	0.41
	1.0	1.43	0.85	1.24	0.52	0.70	0.38	0.51
		1.16		1.0		1.38		1.0
	1.18		1.0		1.36		1.0	
					1.0	1.34		
			1.0	1.46			1.0	1.33

[1] Methylene blue thiocyanate.
[2] Present standard method (2).

environmental conditions as demonstrated by Sherman and Albus (25). Again, changes in the type of flora following preliminary incubation at a lower temperature may result in a more gradual slope of the potential-time curve, as reported by Fay and Aikins (8). In the latter case, the effect would be more marked with the higher dye concentration, which shifts the potential of the zone of decolorization downward and thus increases the time required to reduce the dye.

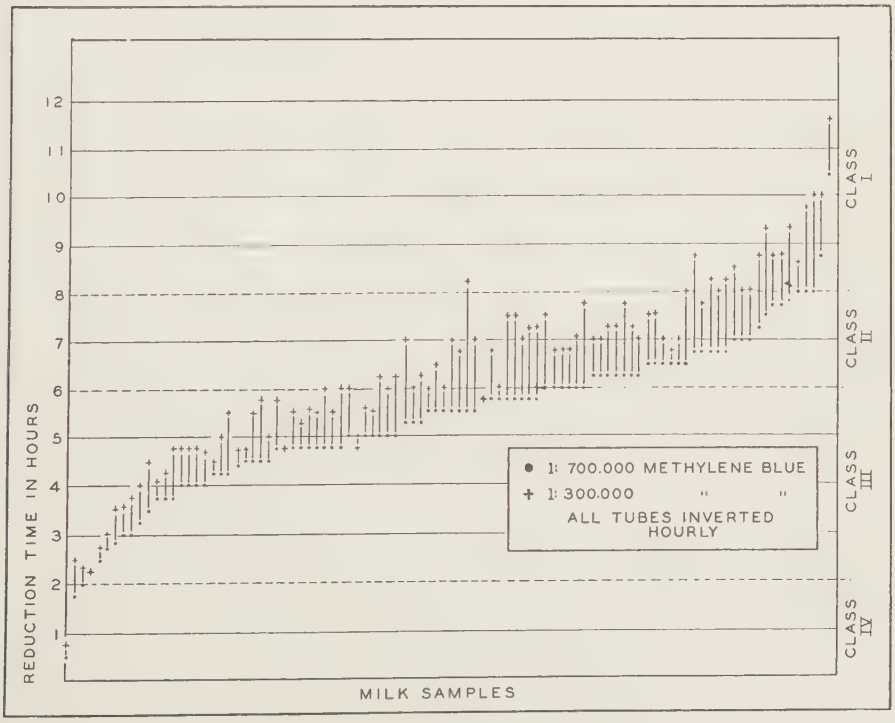

FIGURE 3. Corresponding reduction times of 97 milk samples with old and new dye concentrations.

C. Influence of Mixing Contents of Tubes

The extent to which reduction time is shortened by hourly inversion of the tubes is indicated by the data in Table 5, together with those in Tables 6 and 7, where the samples have been grouped into classes based

TABLE 6.—INFLUENCE OF MIXING UPON REDUCTION TIME AT 37.5° C. NO PRELIMINARY INCUBATION AT 12.8° C.

—	Class	Dye concentration					
		1 : 300,000			1 : 700,000		
		No. of samples	Mixed hourly	Not mixed	No. of samples	Mixed hourly	Not mixed
Average reduction times in minutes	0 – 1 hr.	1	45	47	1	30	30
	1 – 2 hr.	0	—	—	1	105	120
	2 – 3 hr.	2	158	220	4	170	214
	3 – 4 hr.	4	223	269	5	226	288
	4 – 5 hr.	8	277	509	9	279	357
	5 – 6 hr.	9	344	446	7	332	420
	6 – 7 hr.	6	410	473	8	396	521
	7 – 8 hr.	5	456	587	6	471	578
	8 – 9 hr.	4	519	630	1	525	615
	>9 hr.	6	599	773	1	625	900
Averages			377	479		324	411
Relative reduction times	0 – 1 hr.	1	1.0	1.08	1	1.0	1.0
	1 – 2 hr.	0	—	—	1	1.0	1.14
	2 – 3 hr.	2	1.0	1.42	4	1.0	1.26
	3 – 4 hr.	4	1.0	1.21	5	1.0	1.28
	4 – 5 hr.	8	1.0	1.84	9	1.0	1.15
	5 – 6 hr.	9	1.0	1.30	7	1.0	1.26
	6 – 7 hr.	6	1.0	1.15	8	1.0	1.32
	7 – 8 hr.	5	1.0	1.28	6	1.0	1.22
	8 – 9 hr.	4	1.0	1.22	1	1.0	1.17
	>9 hr.	6	1.0	1.29	1	1.0	1.44
Averages			1.0	1.27		1.0	1.27

upon the reduction times of the mixed tubes. Mixing exerts little or no influence upon reduction time of samples reducing in less than 2 hours. This holds true with both dye concentrations, and both with and without preliminary incubation. If samples reducing within 2 hours are excluded, the reduction times for unmixed tubes at both dye concentrations average 35 and 30% greater respectively than those where tubes are inverted. While the variations above and below the mean values for the unmixed tubes tend to cancel each other out where a large enough number of samples are compared, marked variations are evident where the smaller numbers of samples in the different class intervals are compared (Tables 6 and 7).

In order to show more clearly the variable relationship existing between standard and modified reduction times of individual milks, data for 168 milks are presented graphically in Figure 4. Since the modified test, by avoiding the errors resulting from creaming, measures bacterial numbers more accurately than does the standard test (16), the values by the former have been arranged in order of increasing reduction time and the corre-

TABLE 7.—INFLUENCE OF MIXING UPON REDUCTION TIME AT 37.5° C. WITH PRELIMINARY INCUBATION AT 12.8° C.

—	Class	Dye concentration					
		1 : 300,000			1 : 700,000		
		No. of samples	Mixed hourly	Not mixed	No. of samples	Mixed hourly	Not mixed
Average reduction times in minutes	0 – 1 hr.	6	32	35	13	39	39
	1 – 2 hr.	9	94	93	10	105	119
	2 – 3 hr.	12	160	199	10	147	151
	3 – 4 hr.	3	215	325	5	217	346
	4 – 5 hr.	5	280	409	1	255	405
	5 – 6 hr.	2	335	420	1	360	485
	6 – 7 hr.	4	418	578	5	393	522
	>7 hr.	5	494	678	1	500	825
Averages			215	282		156	201
Relative reduction times	0 – 1 hr.	6	1.0	1.10	13	1.0	1.0
	1 – 2 hr.	9	1.0	0.99	10	1.0	1.14
	2 – 3 hr.	12	1.0	1.25	10	1.0	1.03
	3 – 4 hr.	3	1.0	1.51	5	1.0	1.60
	4 – 5 hr.	5	1.0	1.46	1	1.0	1.59
	5 – 6 hr.	2	1.0	1.25	1	1.0	1.34
	6 – 7 hr.	4	1.0	1.38	5	1.0	1.33
	>7 hr.	5	1.0	1.37	1	1.0	1.65
Averages			1.0	1.31		1.0	1.29

NOTE.—In each group the average reduction time with 1 hour mixing is given a value of 1.0, and that for the unmixed tubes is expressed as a multiple of this.

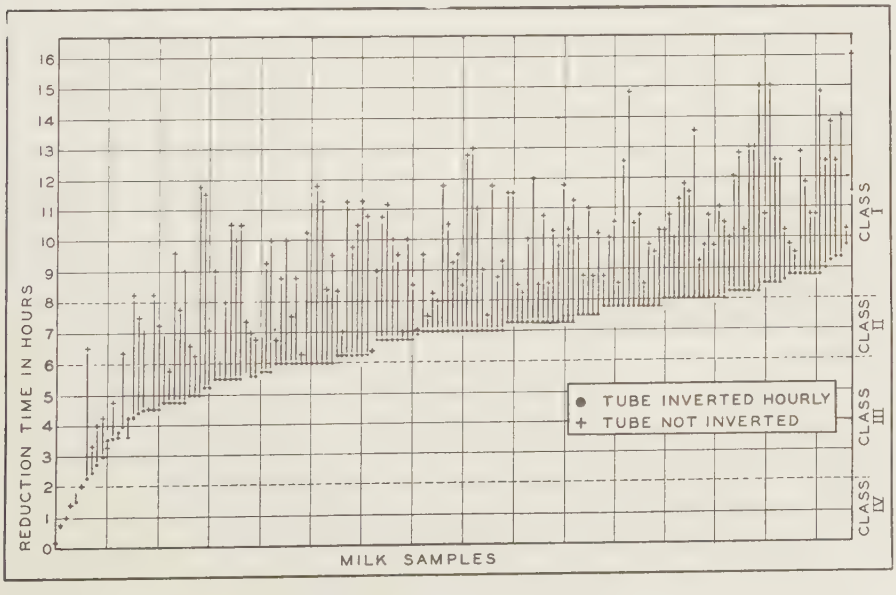

FIGURE 4. Illustrating the variations between reduction times by the standard and modified tests.

sponding values by the standard test plotted against them. The marked fluctuations in reduction time of unmixed samples supplement the recent findings of Frayer (10), who determined comparative reduction times of 50 to 100 tubes by each technique (mixed and unmixed). The wide degree of scatter with the unmixed tubes was in striking contrast to the very close agreement between replicate mixed tubes.

If, as various workers (10, 16, 29, 30, 36) have indicated, the modified reduction test furnishes the more accurate indication of bacteriological condition, then the standard test grades some samples higher and others lower than they should be. In Figure 4 there are 12 samples with modified reduction times of exactly 6 hours. According to the equivalent values suggested by Thornton (34) this corresponds to 8 hours by the standard test. Actually, standard reduction times for these 12 samples vary all the way from $6\frac{1}{4}$ to $11\frac{3}{4}$ hours, with 8 of them in excess of 8 hours. Although such variations have commonly been attributed to the variability displayed by replicate tubes in the standard test, it was discovered that some supplies consistently show wide differences between standard and modified reduction times, while with others the differences are small (16). The available evidence indicates that these differences are related to differences in the rate of creaming and depth of cream layer. Milks which cream rapidly and exhaustively almost invariably take longer to reduce, and are judged by the standard technique to be considerably better than they really are, while with milks which cream poorly the reverse is true. When the mixing modification is employed, the correlation between reduction time and microscopic count of individual bacteria is much superior.

D. Influence of Preliminary Incubation

The present studies on preliminary incubation differ from those reported in 1930 in several respects. At that time, the influence of light upon reduction time (1, 9, 35) was not realized; the dye concentration was approximately 1 : 700,000 (6), while creaming was not interfered with during 37° C. incubation except by a single shaking after 6 hours. In the present studies it has been shown that after preliminary incubation at 12.8° C. for 18 hours, milk is more sensitive to the increased dye concentration, while the optimum temperature is slightly lower than where the test is run without preliminary incubation. However, there is no indication that the value of preliminary incubation is decreased thereby. In the present studies the errors arising from undisturbed creaming have been minimized by hourly mixing, while the influence of light has been excluded.

A study of the data obtained from periodical analysis of milks from 60 shippers supplying a local plant brought to light the interesting fact that certain supplies nearly always showed a much shorter reduction time following preliminary incubation than would be expected on the basis of reduction time of the same sample without preliminary incubation. Field investigations revealed that in every case the morning's milk was held over until the next day before shipment. In Table 8 are presented data from 4 such shippers, along with similar data from 4 others who shipped morning's milk the same day. It seems probable that the bacteriostatic property of the morning's milk was exhausted during the extra 24 hours'

TABLE 8.—VALUE OF PRELIMINARY INCUBATION IN DETECTING MORNING'S MILK HELD OVER AT FARM. MODIFIED REDUCTION TIMES FOR CONSECUTIVE SAMPLINGS

Shipper No.	A.M. milk held over at farm		Shipper No.	A.M. milk shipped same day	
	Reduction time in minutes			Reduction time in minutes	
	A	B		A	B
1	120	15	2	300	240
	150	10		285	175
	360*	220		270	150
	420	120		390	240
	450	195		330	180
	Aver. 300 min.	112 min.		345	210
				375	225
				Aver. 328 min.	203 min.
3	405	260	4	335	135
	225	20		75	10
	480	150		345	175
	495	160		360	230
	420	120		435*	300
	375	225		450	283
	360	90		510	450
	Aver. 394 min.	146 min.		540	420
				Aver. 381 min.	251 min.
5	295	30	6	385	205
	75	10		405	240
	360	0		360	285
	405*	270		315	170
	405	140		420	285
	450	120		375	355
	465	285		360	300
	Aver. 351 min.	122 min.		Aver. 374 min.	263 min.
7	300	30	8	465	210
	360	210		450	300
	285	100		480	315
	225	60		480	375
	360	200		495	465
	240	120		600	540
	Aver. 295 min.	120 min.		Aver. 495 min.	368 min.

A = No preliminary incubation.
B = Preliminary incubation at 12.8° C. for 18 hours.
* = Chlorine sterilization of utensils introduced.

holding at the farm (where the temperature was probably low enough to restrain bacterial growth), so that during preliminary incubation much more active growth took place than in those cases where the milk was fresher. The fact that these differences diminished during the winter months, when the temperature of storage at the farm was low enough to conserve the bacteriostatic property to a greater extent, tends to support this explanation.

In contrast to this, an occasional sample was found which showed a significantly longer reduction time after preliminary incubation. One such sample contained a large number of leucocytes and gave a plate count of 400,000 per ml. on dextrose agar, but only 40,000 per ml. on standard nutrient agar. The plates showed an almost pure culture of streptococci. The reduction times were 130 minutes and 45 minutes respectively with and without preliminary incubation. It seems likely that the shorter reduction time on the fresh sample was due to the activity of leucocytes (26) which were less active after preliminary incubation at a low temperature. Little or no growth of mastitis streptococci would be expected during preliminary incubation, for it has been shown that *Streptococcus agalactiae* fails to form acid at 10° C. (11). Further data relating to the influence of leucocytes and mastitis streptococci are presented in Table 9. These

TABLE 9.—RELATION BETWEEN CELL COUNTS AND REDUCTION TIMES WITH AND WITHOUT PRELIMINARY INCUBATION

Sample No.	Direct microscopic count		Reduction time in minutes	
	Cells	Bacteria[1]	No preliminary incubation	With preliminary incubation
1	1,700,000	100,000*	300	270
2	175,000	25,000	540	570
3	350,000	100,000	525	450
4	950,000	–	420	375
5	2,000,000	–	375	355
6	675,000	50,000	525	450
7	200,000	125,000	525	435
8	350,000	100,000	600	540
9	1,250,000	1,475,000*	300	300
10	2,350,000	450,000*	210	165
11	775,000	300,000*	435	405
12	850,000	< 25,000	555	570
13	1,375,000	150,000*	450	330
14	750,000	33,000	450	390
15	475,000	17,000	480	510
16	1,100,000	417,000	510	465
17	625,000	150,000	585	465
18	800,000	133,000	555	450
19	1,675,000	83,000	450	360
20	750,000	117,000	405	390

[1] Count of individual bacteria (30 fields).
* Long chain streptococci, resembling *Str. agalactiae*, noted.

samples all show less than the average shortening of reduction time following preliminary incubation. While there are a few samples in which cell counts are not particularly high, in most cases they are high enough to be considered abnormal. In many instances, particularly in those where long-chain streptococci resembling *Str. agalactiae* were observed (Nos. 1, 9, 10, 11, and 13), bacteria were sufficiently numerous that were they able to grow actively at 12.8° C., a more marked shortening of reduction time following preliminary incubation would be expected. It would seem, therefore, that where there are large numbers of cells accompanied by few saprophytic organisms, the reduction time following preliminary incubation will be longer than average.

The relative value of the reduction test, both with and without preliminary incubation at the lower temperature, as an index of keeping quality is indicated by the data from 177 samples presented graphically in Figures 5 and 6. In neither case is the correlation as close as that observed in the studies reported in 1930. Two possible explanations for

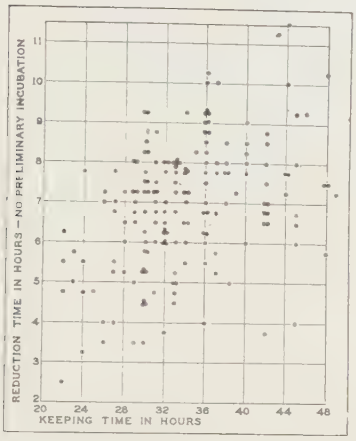

FIGURE 5. Relationship between reduction time (fresh) and keeping quality of 177 samples.

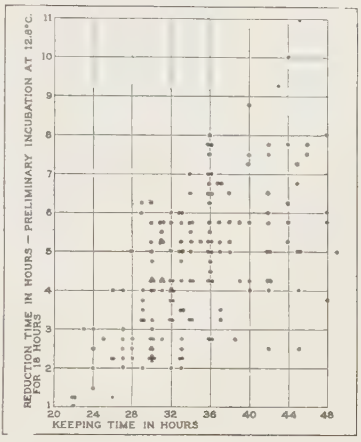

FIGURE 6. Relationship between reduction time (following preliminary incubation) and keeping quality of 177 samples.

these differences suggest themselves. In the 1930 studies, keeping time was determined by organoleptic tests rather than by reduction time of methylene blue at 15.5° C. It may be that the correlation is closer with the former, but there is no theoretical reason apparent which would justify this assumption. In the second place, seasonal differences in flora may be concerned, as the present studies were done on winter milks, while in 1930 summer milks were employed[5].

The correlation between direct microscopic count (individual cells) and keeping time is indicated in Figure 7. Here again the correlation is very much poorer than was obtained in 1930 on summer milks, with a number of high count milks showing unusually long keeping time. The correlation is also definitely poorer than that shown by either of the reduction tests (Figures 5 and 6), suggesting, as in the 1930 studies (15), that the reduction test, especially following preliminary incubation at a lower temperature, furnishes the more accurate estimate of probable keeping quality.

In the 1930 studies (15) the "error score" was adopted as a means of comparing different tests in their ability to rank a series of samples in order of keeping quality. This has also been done in the present studies with four series of samples, the summarized error scores of which are

[5] Data to hand support the view that seasonal differences in flora are responsible, much better agreement with keeping quality being encountered with the coming of warmer weather. This was also observed by Wilson (36).

shown in Table 10. The reduction test following preliminary incubation appears to be much superior to the test on the fresh milk, while both reduction tests are superior to the direct microscopic count. The latter test showed much better agreement with keeping quality during April and May

TABLE 10.—SUMMARY OF ERROR SCORES BY DIFFERENT TESTS (132 SAMPLES)

Date 1938	No. of samples	Total error scores		
		Methylene blue reduction test		Direct microscopic count
		A	B	
Feb. 21	60	638	474	991
Mar 14	20	70	54	100
April 12	24	156	86	156
May 12	28	108	58	112
Total	132	972	672	1359

A = Hourly inversion, no preliminary incubation.
B = Hourly inversion, preliminary incubation for 18 hours at 12.8° C.

than during the 2 preceding months when temperatures were lower. Apparently the flora of certain milks in winter consists mainly of organisms which reduce the dye very slowly.

Preliminary incubation intensifies the difference between good and poor milks. The data in Figure 8 indicate that milks averaging 4 hours

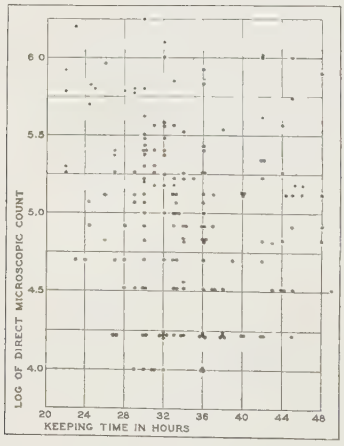

FIGURE 7. Relationship between direct microscopic count of individual bacteria and keeping quality of 177 samples.

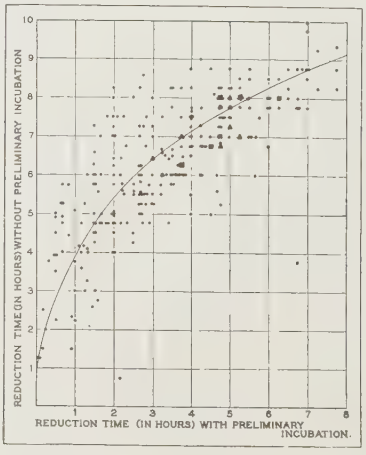

FIGURE 8. Comparative reduction times with and without preliminary incubation.

without preliminary incubation generally decolourize within 1 hour following preliminary incubation, while milks lasting 8 hours with the former will decolourize in around $5\frac{1}{2}$ hours following preliminary incubation. As the reduction time increases beyond this point, the values from the two tests

tend to approach one another. This is to be expected, as the fewer the organisms present, the more nearly the flora approaches that of aseptically drawn milk. Such a flora would not be expected to grow actively at 12.8° C.

Part III. The Effect of Adding the Dye Before Preliminary Incubation

Under practical conditions of testing it would be most convenient if the dye could be placed in the tube before the milk is added. Comparative tests conducted in 1930 showed no significant difference in reduction time whether the dye was added at the start or upon completion of the preliminary incubation. Since in the present studies the stronger dye concentration had been employed and since there was a significantly greater prolongation of reduction time following preliminary incubation (see Table 5), data were obtained on the influence of adding the dye before preliminary incubation.

In the first series of tests, 2 tubes were prepared from each of 20 samples. To one the dye was added at the start of preliminary incubation, while to the other it was added at the end of 18 hours. Both sets of tubes were then shaken vigorously and incubated at 37.5° C. with inversion every half-hour. The average reduction time of the first set was 163 minutes that of the second, 143 minutes, an increase of 14%. In the second series, duplicate tubes were similarly prepared from each of 30 samples at dye concentrations of 1 : 300,000 and 1 : 700,000. Tubes were inverted every hour. With the former the average reduction time was 6% longer, with the latter, 2% longer. With hourly inversion, the difference is so small that the slight delay in reduction where the dye is added at the start has little significance in routine testing.

Part IV. Efficiency of the Methylene Blue Reduction Test Under Winter Conditions

Workers in Scotland and Wales (18, 28) have reported that where samples are held according to the procedure prescribed by the English Ministry of Health (20)[6], an unduly large proportion of winter samples showing plate counts in excess of 500,000 per ml. fails to reduce methylene blue in 5½ hours. Much of the work reported here has been done on winter milks, and temperatures as low as −35° C. have been encountered. Under such conditions milk is often slightly frozen on arrival at the plant. In addition to being held at a low temperature on the farm, a considerable proportion of samples was stored overnight at around 5° C. upon arrival at the laboratory. It would seem, therefore, that such milks were exposed to conditions at least as unfavourable for bacterial growth as would be encountered in Scotland or Wales. Nevertheless, the present studies have failed to disclose any such marked discrepancies between bacterial numbers and reduction times (with or without preliminary incubation at 12.8° C.),

[6] Milk samples must be kept at atmospheric (shade) temperature until 6 p.m. on the day of production if from a morning milking, and until 10 a.m. on the next day if from an afternoon milking.

as the data from 18 representative samples in Table 11 indicate[7]. In addition to the direct microscopic counts of individual bacteria, the samples were plated on 1% milk agar after having been held at approximately 4° C. for 28 hours after arrival at the laboratory. It will be seen that the maximum plate count obtained was under 200,000 per ml. and the maximum

TABLE 11.—RELATIONSHIP BETWEEN BACTERIA COUNTS AND REDUCTION TIMES OF WINTER MILKS

Sample No.	Reduction time, minutes		Direct microscopic count		Plate count (milk agar) 48 hours at 37° C.	Keeping time[2] hours
	A[1]	B	Bacteria	Leucocytes		
5	435	240	133,000	750,000	25,500	26
8	435	345	67,000	250,000	15,500	34
48	435	240	67,000	1,225,000	68,500	30
11	420	255	50,000	450,000	66,000	39
30	420	255	333,000	500,000	53,500	34
44	420	195	100,000	475,000	55,500	30
49	420	300	50,000	1,225,000	22,500	36
53	420	255	17,000	1,750,000	54,500	32
14	405	300	83,000	450,000	66,000	39
55	405	255	250,000	750,000	47,500	32
37	390	195	550,000	100,000	44,000	32
47	390	150	83,000	150,000	74,000	28
22	375	225	100,000	650,000	90,500	32
28	375	240	17,000	700,000	37,000	32
41	375	225	367,000	1,150,000	163,000	32
15	360	240	133,000	600,000	42,000	40
20	360	195	383,000	350,000	192,500	32
21	360	195	183,000	475,000	121,500	34

[1] A = Reduction time without preliminary incubation.
B = Reduction time following preliminary incubation at 12.8° C. for 18 hours.
[2] Reduction time upon incubation at 15.5° C.

microscopic count 550,000 per ml. The reduction times are in good agreement with the counts, indicating no marked inhibition of bacterial activity during incubation at 37° C. as a result of prolonged holding at low temperature.

To throw further light upon the differences in findings just referred to, the following experiment was conducted. An 8-gallon shipping can was rinsed with a sample of mixed milk held at 4° C. for 8 days. After rinsing with tap water, the can was allowed to stand with the lid on at around 22° C. for 21 hours, when it was rinsed with 1 litre of sterile water. A quantity of fresh raw milk from the morning milking was cooled at once, divided into 3 portions and duplicate tubes treated as indicated in Table 12. At the end of the holding periods indicated, the dye was added and tubes incubated at 37.5° C. with hourly inversion. The results indicate that in the case of rapidly cooled milk, tested immediately or held at a low temperature for 24 hours, the reduction time may be much greater than would be expected from the corresponding counts. Presumably this is due to the bacteriostatic property of the milk being active in both the fresh portion

[7] Of 272 samples examined between Dec. 28, 1937 and March 14, 1938, only 6 with direct microscopic counts in excess of 500,000 bacteria per ml. have failed to reduce the dye within 5½ hours. The maximum count observed was 1,400,000, which would be equivalent to a plate count of 350,000 per ml. (2).

TABLE 12.—INFLUENCE OF VARIOUS TREATMENTS UPON REDUCTION TIME OF MILK INOCULATED WITH CAN RINSINGS

Sample portion	Reduction time in minutes (duplicate tubes)				Bacteria count at start		Keeping time in hours
	1	2	3	4	Direct microscopic count	Standard plate count	
A	180	645	660	>480	50,000	29,800	75
B	75	465	345	465	550,000	190,000	48
C	45	330	195	330	2,417,000	2,200,000	40

A = Control portion; B = 1% can rinse added; C = 10% can rinse added.
1 = Held at 22° C. for 12 hours, then placed in refrigerator at 4° C. for 12 hours.
2 = Held at 4° C. for 24 hours.
3 = Held at 4° C. for 6 hours, then given preliminary incubation at 12.8° C. for 18 hours.
4 = Reduction test at 37.5° C. run at once.

and also in the portions cooled at once. Where the milk stood at room temperature for 12 hours before being placed in the refrigerator (No. 1), the bacteriostatic property was evidently diminished, as indicated by the much shorter reduction times when tested after subsequent storage at low temperature. It may be that the disparity between the results obtained here with the ordinary run of raw milks and those reported from Scotland and Wales is due to the more rapid cooling of the milks examined by these workers, with a consequent greater conservation of the bacteriostatic property (14). Furthermore, the samples studied here (exclusive of those reported in Table 12) represented the mixed milk from both morning's and evening's milkings, while those studied in Britain appear to have been from individual milkings. The bacteriostatic activity in the latter might be expected to be significantly greater than in the former.

DISCUSSION

The present studies have shown that for all practical purposes it makes little difference whether tubes are inverted at $\frac{1}{2}$, 1 or 2 hour intervals. By inspection and inversion every 2 hours, samples could be placed in the appropriate grades on the basis of three observations—after 2, 4 and 6 hours. The relation of these reduction times to those by the standard technique is depicted in Figure 9. Approximately equivalent values for the standard and modified techniques, and for the latter following preliminary incubation, are as follows:

No preliminary incubation		Preliminary incubation at 12.8° C. for 18 hrs. Tubes mixed every 2 hrs. (hours)
Tubes not mixed (hours)	Tubes mixed every 2 hours (hours)	
11	$7\frac{1}{2}$	$4\frac{1}{2}$
$8\frac{1}{2}$	6	$2\frac{1}{2}$
5	4	1
2	2	$\frac{1}{4}$

Where interest is confined to the detection of the poorest grades of milk, mixing may be dispensed with, since it has little effect on milks reducing within 2 hours (Figure 9 and Tables 6 and 7). On the other hand, the bacteriological quality of the more satisfactory grades of milk can be determined with a much greater degree of reliability where the "creaming error" is minimized by periodical inversion of the tubes.

The use of the stronger dye concentration lengthens the reduction time to a significantly greater extent than reported by Thornton and Sandin (31)[8]. As against this must be set the greater ease with which reduction can be detected. (The lack of depth of colour with the weaker dye concentration has given rise to serious trouble in some areas (31).) The increase in reduction time following the use of the higher dye concentration results in a more lenient grading of the milk. This appears to furnish a further reason for adopting the mixing modification. With few exceptions, the shortening of reduction time resulting from regular dispersion of the cream layer more than compensates for the increase in reduction time due to the stronger dye concentration, as indicated by the data from 87 samples presented in Figure 10, where the present standard technique with the old dye concentration is compared with the modified technique (hourly mixing) and the new dye concentration. With the adoption of the modified technique, therefore, grading would generally be more severe instead of more lenient.

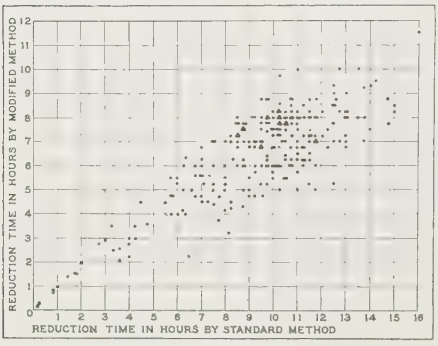

FIGURE 9. Comparative reduction times by standard and modified (one hour) techniques.

The view has been expressed (29) that the standard reduction test (with the old dye concentration) is not sufficiently accurate beyond the $5\frac{1}{2}$ hour limit. With the new dye concentration, which extends reduction time by approximately 20% (Table 5), a considerably greater percentage of samples will exceed the limit of accuracy suggested, and as milk supplies continue to improve, this will approach 100%. The proposal of the American Public Health Association to raise the limit for Class I milk from $5\frac{1}{2}$ to 8 hours (5) suggests that in many districts interest now extends to the better grades of milk. If the test is to be used over periods extending to 8 hours, steps should be taken to improve its accuracy and reliability by the simple expedient of periodical inversion of tubes.

Further experience has confirmed the value of preliminary incubation at 12.8° C. for 18 hours. This procedure encourages the growth of saprophytic organisms concerned with milk spoilage, and thus intensifies the difference between really clean milks (where the flora is mainly from the udder) and those heavily contaminated with saprophytes during production

[8] While Thornton and Sandin believe the greater depth of colour may be the main factor in increasing the reduction time with the increased dye concentration, it seems more likely to be due to the downward shift of potential of the zone of reduction (8) together with increased bacteriostasis.

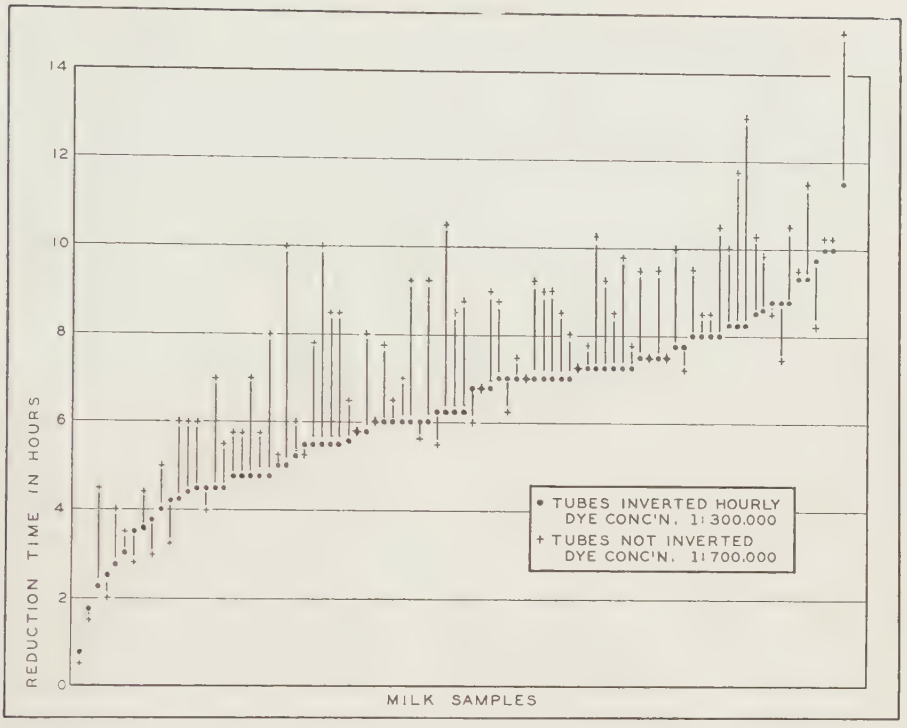

FIGURE 10. Comparative reduction times with old dye concentration (unmixed) *versus* new dye concentration (hourly mixing).

and handling. In consequence, reduction times following preliminary incubation are in better agreement with keeping quality than where preliminary incubation is omitted. This procedure also enables the detection of inadequately cooled milk held over on the farm until the following day.

From the standpoint of convenience to the operator, the preliminary incubation has much to commend it. Since the average milk lasting 8 hours with the present standard technique reduces in approximately $2\frac{1}{2}$ hours following preliminary incubation, the time required to classify a series of samples is greatly reduced. It also permits the starting of the test at the most convenient hour.

SUMMARY

1. The average optimum temperature of incubation increases slightly with increasing reduction time, but is close to 37.5° C. Holding samples for 24 hours at 5° C., or for 18 hours at 12.8° C., causes a slight downward shift but not sufficient to warrant changing the present standard temperature.

2. The modified test, wherein the cream layer and accompanying bacteria are dispersed by periodical inversion, is preferable to the standard test. Variability is reduced, reduction time is generally shortened and decolorization is more uniform, especially with the better grades of milk.

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3. Milks showing similar reduction times by the modified technique may show widely different standard reduction times. These discrepancies appear to be associated with differences in the degree and rate of creaming. This is believed to be a serious objection to the use of the standard technique with the better grades of milk.

4. Inversion of tubes at 2-hour intervals has yielded results in good agreement with those from more frequent inversion. The adoption of class limits of 2, 4 and 6 hours, with inversion of tubes at these intervals, is recommended as increasing the reliability of the test with a minimum expenditure of time and effort.

5. Reduction time is lengthened by approximately 20% when the dye concentration is increased to 1 : 300,000. This is more than compensated for (in all but the poorer grades of milk) by the shortening of reduction time when tubes are inverted periodically.

6. Preliminary incubation at 12.8° C. for 18 hours improves the test as an index of keeping quality and brings about a significant shortening of reduction time, particularly with the lower grades. This modification accentuates the differences between good and fair quality milks, and has proven valuable in detecting certain faulty farm practices.

7. Promptly cooled milk may show reduction time and keeping time considerably longer than might be expected on the basis of initial bacterial content. This is probably due to preservation of the bacteriostatic principle. From the standpoint of indicating keeping quality, the sensitivity of the reduction test to growth-restraining influences is an advantage not possessed by counting methods.

ACKNOWLEDGMENTS

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BROWN ROT OF PEACHES IN TRANSIT AND STORAGE[1]

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Brown rot, caused by *Sclerotinia fructicola* (Wint.) Rehm., is of such outstanding importance in practically every peach-growing district of the world, that it is almost trite to remark that the Ontario peach industry has endured heavy losses from the disease in the orchard, during transportation, on the market, and even in the hands of the consumer. Most of the investigations on brown rot in the past have been concerned mainly with control in the orchard, and rightly so, because failure there is of primary importance. The present studies, on the contrary, have been directed chiefly towards acquiring information concerning the effectiveness of control measures and the behaviour of the disease after the fruit is harvested, for, very often, while there may be little or no rot evident in the orchard, considerable may develop in the course of long distance transit, for example to the Canadian West, and even in the shorter hauls to nearby points.

REVIEW OF LITERATURE

The literature on brown rot and its control is so voluminous that no attempt will be made in the present instance to deal exhaustively with the subject, a detailed and comprehensive discussion of which may be found in the bulletin of Roberts and Dunegan (19). The consensus of opinion (7, 12, 18, 19, 20) is that effective orchard control can be achieved by careful sanitary measures and a series of summer sprays. Sanitation involves the removal and destruction of blighted twigs and mummied fruits. For the most part, the spray programmes are based on the schedule introduced by Scott (21), and comprise a pre-blossom spray of wettable sulphur, or lime-sulphur 1 : 40; a "shucks" spray of self-boiled lime-sulphur or wettable sulphur,with poison where necessary for the control of curculio, and a spray or dust, without poison, applied three or four weeks before harvest. However, to this programme there are a number of variants in both materials and timing (6, 7, 9, 12, 14, 16, 17, 18, 19, 25). According to Snapp, Alden *et al.*(17) spraying gave more effective control than dusting.

Several investigators (2, 4, 5, 10, 22) have expressed the opinion that rots developing in transit and in the market may be considerably reduced by spraying or dusting. Brooks and Cooley (3) state that, while brown rot develops at a lower temperature on peaches than on potato dextrose agar and at a lower temperature on ripe peaches than on green, its development is delayed longer the nearer the temperature approaches freezing. In a later paper, these authors (4) indicate that low temperature had a relatively greater inhibitory effect upon the development of rot during the incubation period than during later growth. For that reason there was

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Work carried out in co-operation with the Fruit and Vegetable Products Division, Marketing Service, Dominion Department of Agriculture, Ottawa.

[2] Plant Pathologist.

greater damage from rot when cooling was delayed, than when cooling was effected immediately after harvest, especially in inoculated peaches. They also found that both the temperature and rate of cooling in refrigerator cars varied greatly from place to place in the car, and from the top to the bottom layers of boxes. To secure greater uniformity of temperature in iced cars, Brooks (2) suggested precooling, the use of car blowers, or the addition of salt or dry ice to the ice in the bunkers. Hienton and Fawcett (15) advocate that salt be added to the ice, and that peaches be packed in the car in such a way, and in such containers, that the air may circulate freely about the fruit.

Brooks (2) found a positive correlation between shipping losses in Georgia peaches and the rainfall during the growing season in the case of brown rot, but not when *Rhizopus nigricans* was involved. Anderson (1) and Fish (10) consider *R. nigricans* to be of greater importance than *S. fructicola* as a transit rot. Moreover, *R. nigricans* is not amenable to control by spraying (1, 10) but seems to be dependent on picking, packing and shipping conditions rather than on factors operative in the orchard (2, 10). *R. nigricans* is more readily checked by low temperatures than is *S. fructicola* (3) and can be reduced by sanitation in picking and packing and by sterilizing the equipment (10).

The conclusion of Dorsey and Potter (8) that brushing or "defuzzing" peaches would facilitate infection by allowing spores to make more direct contact with the surface of the fruit, was confirmed by the experimental work of Smith (26). Smith (24) also demonstrated that dusting-sulphur applied before inoculation with fresh spores of *S. fructicola* was effective in controlling brown rot in both brushed and non-brushed peaches held at 45 to 55° F., but not at 65° F. or higher.

MATERIALS AND METHODS

Peaches of the varieties Rochester and Elberta were used throughout the experiments. The orchards were divided into experimental plots according to the fungicide used. The procedures followed during picking and packing operations and in the taking of observations in the years 1933 to 1937, were formulated as the result of some preliminary trials in 1932. Fruit from each plot was picked in the morning and immediately taken to the packing house to be sorted by visual inspection into three grades, (i) "green" in which the fruit was mature enough to ripen at room temperature, but had not yet changed appreciably in colour; (ii) "intermediate" in which the ground colour was beginning to take on the yellow shades; (iii) "ripe" in which the ground colour was definitely yellow and the fruit was beginning to soften but was not yet ripe enough to be eaten out of hand. As the sorting proceeded, the peaches were wrapped in tissue paper and packed in 2-layer lugs, so that packing was completed on the day of harvest. Throughout these operations the peaches were carefully handled, except in specified instances, in order to minimize bruising or puncturing. Care was also taken to avoid handling rotting fruits while collecting the experimental samples. Peaches from each lot were tested for firmness by means of a pressure-tester, and in the first 2 years their ground and flesh colour were recorded after comparison with Ridgeway's colour chart.

Shipments, comprising one or two boxes of each lot, were sent in iced cars at a temperature of 45° to 50° F., to Winnipeg or to Saskatoon, distances of 1,000 and 1,500 miles respectively. In most cases, duplicates of these shipments were held for examination at St. Catharines. Each year also, a transatlantic consignment of Elberta peaches was shipped to England via port of Montreal.

Observations were taken at intervals of 3 or 4 days over a period of 2 to 3 weeks after picking in the case of lots examined at St. Catharines, and at similar intervals for a week or 10 days after arrival in the case of shipments. The peaches were handled in the wraps and not touched directly by hand during the various examinations, at each of which any fruits showing spots of rot were recorded and discarded, regardless of the extent of the rot. These methods were employed to avoid, as far as possible, both contamination during handling and undue spread of rot by contact during the intervals between examinations.

OBSERVATIONS

The graphs (Figures 1 to 5), which present the data obtained from the various lots or shipments, although apparently 3-dimensional in form, should really be considered as multiplanar. Each consists of two intersecting sets of parallel planes, arranged to facilitate the comparison of 2 sets of interacting conditions or factors. Dates of spraying and of precipitation during the growing seasons from 1933 to 1937 are given in Figure 6.

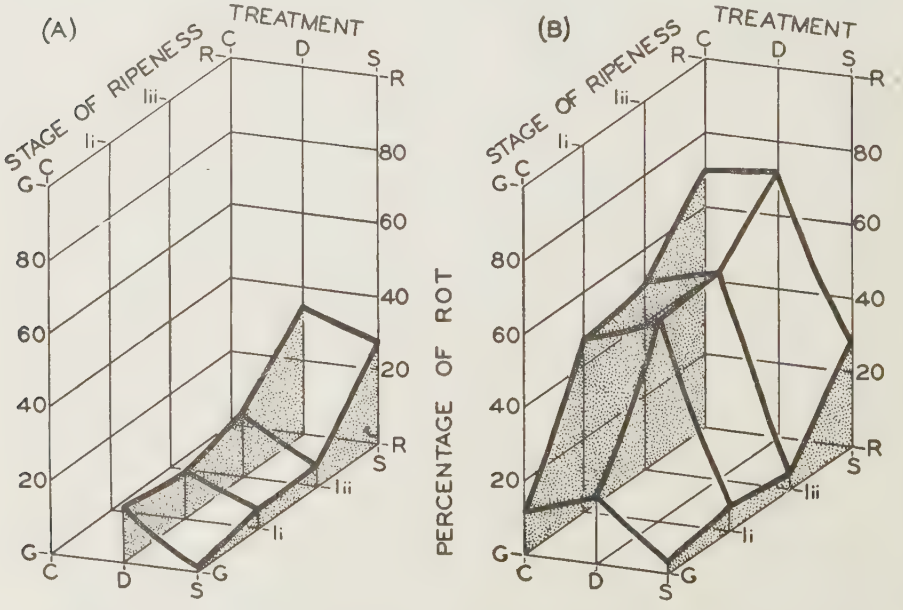

FIGURE 1. Incidence of rot, in relation to treatment and stage of ripeness at harvest, in 1933 in Rochester peaches: (A) examined at Winnipeg; (B) at St. Catharines. Stages of ripeness: G, greenest grade of pick; Ii, Iii, intermediate; R, ripest; all except Iii carefully handled. Treatment: C, check; D, dusted; S, sprayed with Koloform. See also Table 1.

Experiments in 1933

In the first pick of Rochester (Figure 1, A) the checks were omitted from the record as not comparable with the treated peaches, for not only were they taken from a different orchard but also an unsuccessful attempt was made to sort them during picking. In the second pick (Figure 1, B), while the dusted peaches were from the same source as in pick 1, and the checks and sprayed peaches were from different orchards, the samples were more nearly alike, as indicated by the pressure tests. That the percentage of rot in the sprayed peaches of the second pick on the fourth day at room temperature was about the same as that of similar fruit of the first pick on the second day, was largely due to the lower temperature during the holding period for the second pick (Table 1). The failure of the dust in the second pick was undoubtedly due to the heavy rainfall immediately preceding harvest (Figure 6). The rain evidently had little effect on the spray.

TABLE 1.—DATA RELEVANT TO PICKS OF ROCHESTER PEACHES IN 1933 (SEE ALSO FIGURE 1)

Reference in Fig. 1	Date of		Observations			Room temp., °F.*	Remarks
	Pick	Arrival	Date	Days after harvest	Days at room temp.		
A	Aug. 16	Aug. 21	Aug. 23	7	2	72–80 (78)	Shipped to Winn. Aug. 16.
B	Aug. 18	Aug. 26	Aug. 30	12	4	62–72 (68)	Held at 33–36° F. till Aug. 26.

* Range and estimated mean (in brackets) of temperature during the period indicated in the previous column.

With the possible exception of the third pick of Elberta, held in the slightly damper atmosphere of the cellar (Figure 2, C; Table 2), dust and spray gave approximately the same degree of control and both showed considerable improvement over the checks. It should also be noted that transportation to Saskatoon (Figure 2, A) in a properly iced car had little

TABLE 2.—DATA RELEVANT TO PICKS OF ELBERTA PEACHES IN 1933 (SEE FIGURE 2)

Reference in Fig. 2	Date of		Observations			Room temp., (°F.)	Remarks
	Pick	Arrival	Date	Days after			
				Picking	Arrival		
A	Sept. 8	Sept. 14	Sept. 21	13	7	57–80 (65)	All but Iiii precooled; shipped to Saskatoon Sept. 9.
B	Sept. 5	Sept. 9	Sept. 13	8	4	66–70 (68)	Shipped to Winnipeg Sept. 5.
C	Sept. 11	Sept. 11	Sept. 18	7	7	58–70 (65)	In cellar at St Catharines.
D	Sept. 11	Sept. 11	Sept. 18	7	7	60–80 (70)	In laboratory at St Catharines.

or no ill effect on the fruit, since the amount of rot after a week at room temperature and almost 2 weeks after harvest compared favourably with that in the lots examined at St. Catharines (Figure 2, C, D) after a week

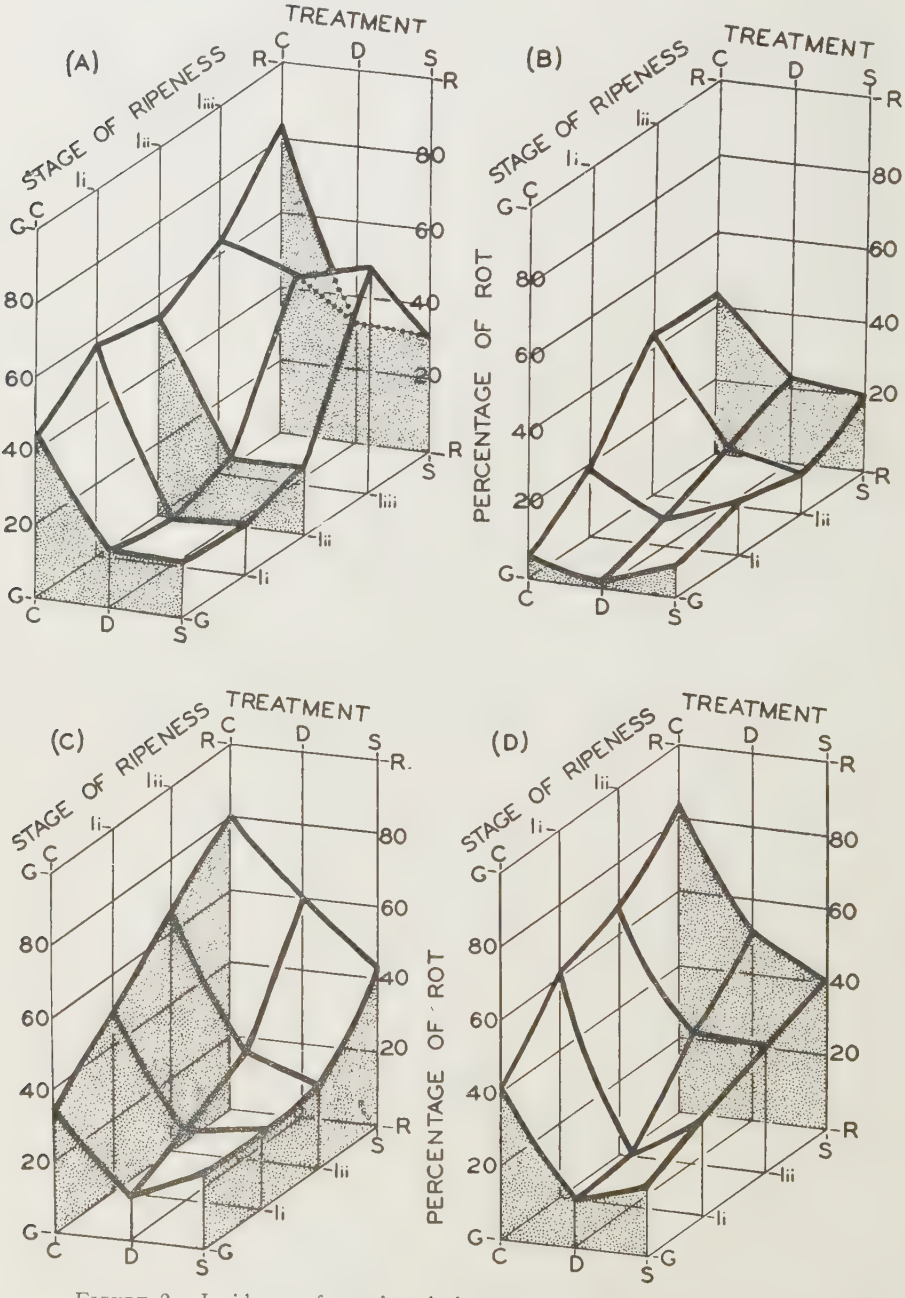

FIGURE 2. Incidence of rot, in relation to treatment and stage of ripeness at harvest, in 1933 in Elberta peaches: (A) examined at Saskatoon; (B) at Winnipeg; (C) and (D) at St. Catharines. Stages of ripeness and treatments as in Figure 1. See also Table 2

at room temperature and only one week after harvest, except where there was a 24-hour delay in cooling (Iiii, Figure 2, A).

Experiments in 1934

In and after 1934 a wider variety of spray materials was used, mainly for the purpose of comparing the more recently introduced pre-harvest sprays, such as Flotation sulphur, Bartlett's pre-pick, etc., with the older wettable sulphurs such as Koloform, etc. which have to be applied at a longer interval before harvest because of their tendency to mark the fruit.

The 1934 Rochester shipments (Figure 3, A) demonstrate not only the relative efficiency of the several fungicides under different conditions, but also the importance of those conditions. Neither shipment received the best treatment in transit, since the one, the day after picking, went in an iced car to Brandon and thence uncooled to Winnipeg, while the other, immediately after packing, went in an iced car to Yorkton and thence, uncooled to Saskatoon. In spite of this treatment, the latter, except for the checks, was in a satisfactory condition a week after arrival, while the former had approximately the same rot development as the lot held at St. Catharines about the same number of days after harvest, but 8 days longer at room temperature (Table 3). The higher incidence of rot in the Winnipeg shipment may have been in some measure due to the rain which occurred the day before picking (Figure 6) but, in the light of the 1933 experience, the delay in getting the fruit cooled (Table 3) was, in all probability, mainly responsible.

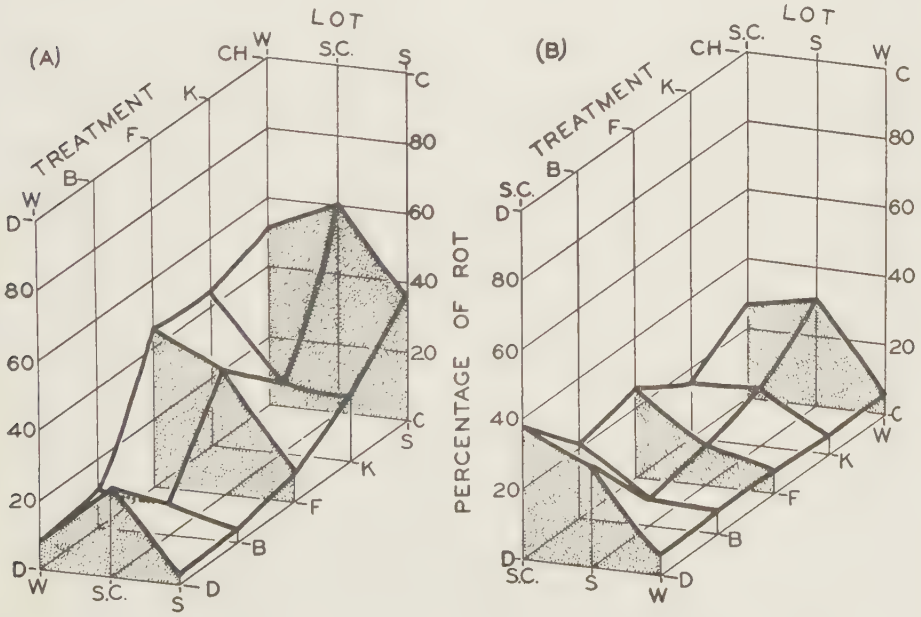

FIGURE 3. Incidence of rot, in relation to treatment and post-harvest conditions in 1934: in (A) Rochester peaches, intermediate stage of ripeness; (B) Elberta peaches, three stages of ripeness combined. Treatments: C, check; K, sprayed with Koloform; F, with Flotation sulphur; B, with Bartlett's pre-pick; D, dusted with sulphur. Lots: SC, examined at St. Catharines; W, at Winnipeg; S, at Saskatoon. See also Table 3.

TABLE 3.—DATA RELEVANT TO THE PICKS OF ELBERTA AND ROCHESTER PEACHES IN 1934 (SEE FIGURE 3)

Reference in Fig. 3	Date of		Observations			Room temp. (°F)	Remarks
	Pick	Arrival	Date	Days after			
				Harvest	Arrival		
Rochester							
A (W)	Aug 20	Aug. 29	Sept. 4	15	6	58-70 (62)	Put in iced car Aug. 21, for Winnipeg, via Brandon.
A (S.C.)	Aug. 22	Aug. 22	Sept. 5	14	14	60–76 (65)	Held at St. Catharines.
A (S)	Aug. 22	Aug. 31	Sept. 7	16	7	56-70 (61)	Put in iced car for Saskatoon via Yorkton, Aug. 22.
Elberta							
B (S.C.)	Sept. 13	Sept. 13	Sept. 27	14	14	58–78 (66)	Papers damp in dusted lots. Humidity very high shortly after harvest.
B (S)	Sept. 13	Sept. 23	Oct. 1	18	8	50–71 (65)	Shipped to Saskatoon via Regina, Sept. 13.
B (W)	Sept. 10	Sept. 14	Sept. 21	11	7	59–72 (66)	Shipped to Winnipeg Sept. 10. Car iced only at loading.

The shipment of Elberta peaches to Saskatoon underwent treatment almost identical with that of the corresponding Rochester shipment, and, except for the dusted lots, with much the same results (Figure 3, B; Table 3). The failure of the dust to control brown rot was probably due to the intervening rains (Figure 6). In the case of the Winnipeg consignment, as the car was iced only once at loading and not en route, the temperature during the latter part of the journey was undoubtedly higher than could be desired. It is also exceedingly probable that, because of the melting of the ice and the influx of warmer air, the relative humidity in the car was abnormally high during the same period. The 1935 Elberta shipment to Winnipeg (Figure 4, B; Table 4) travelled under similar conditions, except that it was loaded into the refrigerator car two days after harvest. Since, in both instances, there was little difference in the amount of rot in the various samples it is suggested that prolonged periods of high relative humidity tend to render the fungicides ineffective. This suggestion receives a certain amount of support from the behaviour of the Elberta peaches held at St. Catharines in 1934 (Figure 3, B) which were subjected to very high humidity during the first few days after harvest (cf. Figure 6).

Experiments in 1935

So far as the various lots of Rochester peaches were concerned (Figure 4, A) there was little to choose between the different fungicides employed, as the disease was evidently easy to control in 1935. A comparison of the

checks in the lots, both of Rochester (Figure 4, A) and of Elberta (Figure 4, B) held at St. Catharines would indicate that the second pick has a tendency to be more susceptible to rot than the first. In spite of this increased susceptibility, the incidence of rot was considerably higher in the Rochester shipment to Saskatoon than in that to Winnipeg, as a result of the delay between picking and cooling in the former (Table 4.) The difference is all the more striking if the strictly comparable lots are considered together, that is, the Winnipeg consignment and the second pick at St. Catharines on the one hand, and the Saskatoon shipment and first pick at St. Catharines on the other (Figure 4, A). The same effect appears again in the picks of Elberta (Figure 4, B).

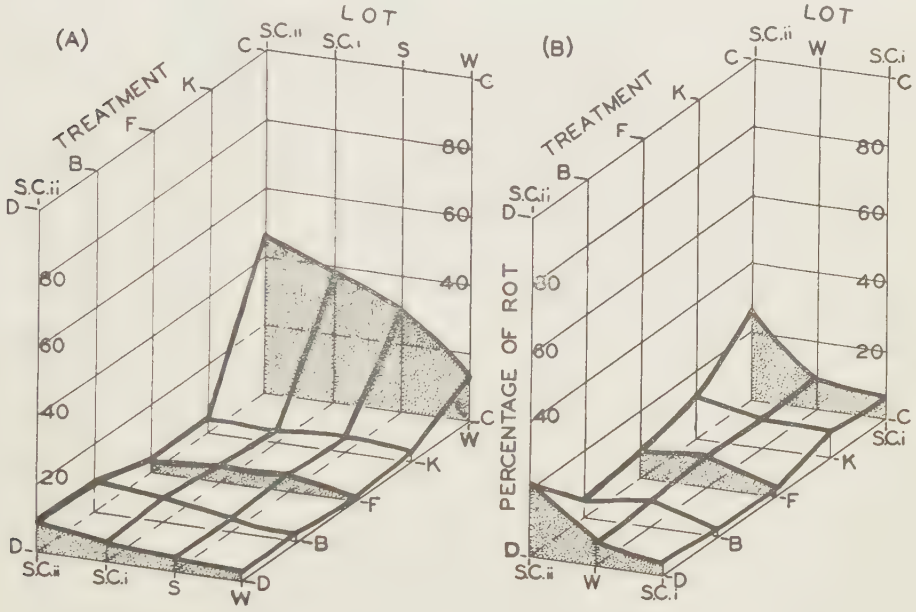

FIGURE 4. Incidence of rot, in relation to treatment and post-harvest conditions in 1935: in (A) Rochester peaches, intermediate stage of ripeness; (B) Elberta peaches, three stages of ripeness combined. Treatment and lots as in Figure 3. See also Table 4.

Experiments in 1936

Because of the comparative drought throughout the growing season of 1936 (Figure 6) brown rot was of such minor significance in that year that no conclusions could be drawn from the experiments, beyond the obvious one that there is a marked positive correlation between the incidence and severity of brown rot and precipitation during the growing season, even when the rainfall is high only in the early summer (cf. 1935, 1936 and 1937, Figure 6).

Experiments in 1937

The incidence of rot in relation to the time factor and to treatment is illustrated in Figure 5. These curves are typical of those obtained in previous years when the amount of rot appearing at successive observations

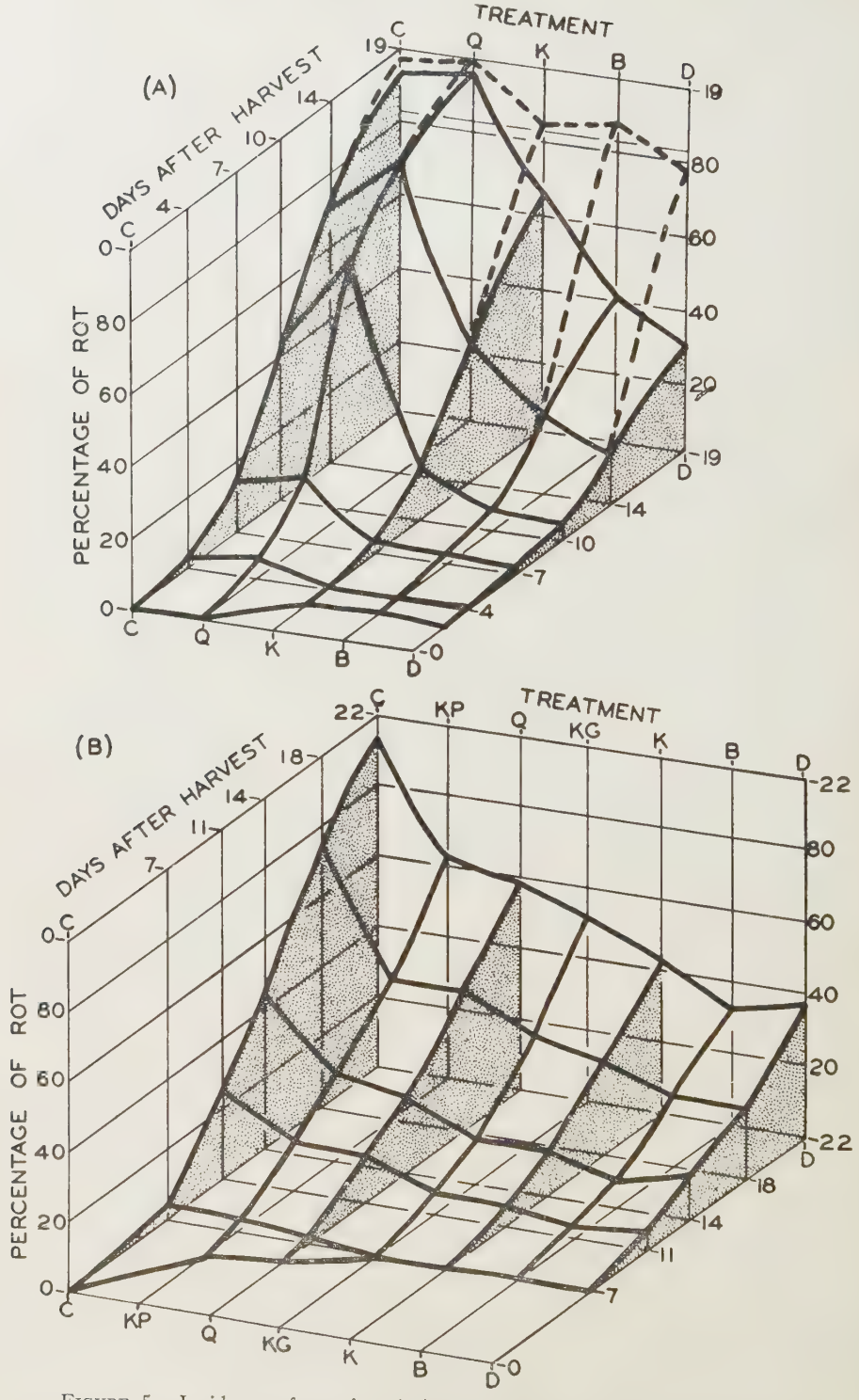

FIGURE 5. Incidence of rot, in relation to treatment and the time-factor, in 1937: in (A) Rochester peaches; (B) Elberta peaches. Treatments as in Figures 3 and 4, with the addition of KG, Kolofog extra; KP, Kolopick, and Q, Qua-sul. See also Table 5.

TABLE 4.—DATA RELEVANT TO THE PICKS OF ROCHESTER AND ELBERTA PEACHES IN 1935 (SEE FIGURE 4)

Reference in Fig. 4	Date of		Observations			Room temp., (°F.)*	Remarks
	Picking	Arrival	Date	Days after			
				Harvest	Arrival		
Rochester							
A (SCii)	Aug. 29	Aug. 29	Sept. 12	14	14	58, 60, 64, 61, 60 (60)	Second pick held at St. Catharines.
A (SCi)	Aug. 27	Aug. 27	Sept. 10	14	14	64, 58, 63, 62, 60 (61)	First pick, held at St. Catharines.
A (S)	Aug. 27	Sept. 3	Sept. 10	14	7	65, 64, 63 (64)	In iced car Aug. 28 for Saskatoon.
A (W)	Aug. 29	Sept. 5	Sept. 12	14	7	66, 61, 61 (61)	In iced car Aug. 29 for Winnipeg.
Elberta							
B (SCii)	Sept. 20	Sept. 20	Oct. 3	13	13	63, 60, 56, 49, 49 (56)	Second pick held at St. Catharines.
B (W)	Sept. 17	Sept. 25	Sept. 30	13	5	63, 68 (66)	In iced car Sept. 19 for Winnipeg, car iced once only.
B (SCi)	Sept. 17	Sept. 17	Sept. 30	13	13	67, 63, 60, 56, 49 (60)	First pick held at St. Catharines.

* Mean temperature of successive 3-day intervals; (mean temperature for full period in brackets).

was plotted against time elapsed after harvest. In the curves for the Rochester variety (Figure 5, A) the broken lines indicate the total number of peaches affected with rot in each lot, while the solid lines include adjustments to allow for "nesting" and for the secondary rots accompanying senile breakdown. The solid line, then, represents individual infections, chiefly those of brown rot, though including those caused by *Rhizopus nigricans*.

While it is true that there was a markedly higher percentage of rot in Rochester peaches 19 days after harvest than there was in Elberta after 18 days, a study of the temperature obtaining during the respective holding periods (Table 5) would suggest that the incidence of rots in these 2 varieties is influenced as much by meteorological conditions just before and after harvest as by inherent differences in susceptibility, which may be less than is generally supposed.

ENGLISH SHIPMENTS

Except in 1933, the peaches consigned to England were packed in wood wool in single layer boxes wired in pairs. This method of packing greatly reduced damage from bruising in transit. Usually the packs were expressed to Montreal and there transferred to the refrigerated hold of the ship, where they were kept at a temperature of 33° to 36° F. until arrival at London or Liverpool.

TABLE 5.—DATA RELEVANT TO PICKS OF ROCHESTER AND ELBERTA PEACHES IN 1937, EXAMINED AT ST. CATHARINES (see FIGURE 5)

Reference in Fig. 5	Date of pick	Mean daily temperature (°F.), (approximate temperature of packing house)																						Mean
		Days after harvest																						
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
(Rochester) A	Aug. 20	78.3	78.0	66.6	64.0	67.5	68.9	71.6	74.9	74.0	70.9	74.1	75.6	78.8	79.7	81.2	75.6	61.9	56.2	62.0	69.2				71.5
		74.3			66.8			73.5			73.5			79.9			64.5			65.6				
(Elberta) B	Sept. 16	57.2	52.0	54.3	54.2	50.7	55.4	55.3	64.4	72.4	75.1	58.3	49.3	49.6	54.4	52.8	66.4	56.8	50.1	55.6	63.1	71.8	54.3	42.8	57.2
		54.5			53.5			64.0			60.9			52.3			57.8			63.5			48.5	

The results need not be discussed in detail as in most years very little rot occurred in any lot except the check, so that, except in 1937 when the data agreed very well with those obtained in St. Catharines, it was not possible to arrive at any conclusion concerning the relative efficiency of the fungicides used. The chief interest in the shipments to England was the demonstration that, with proper treatment and careful handling, brown

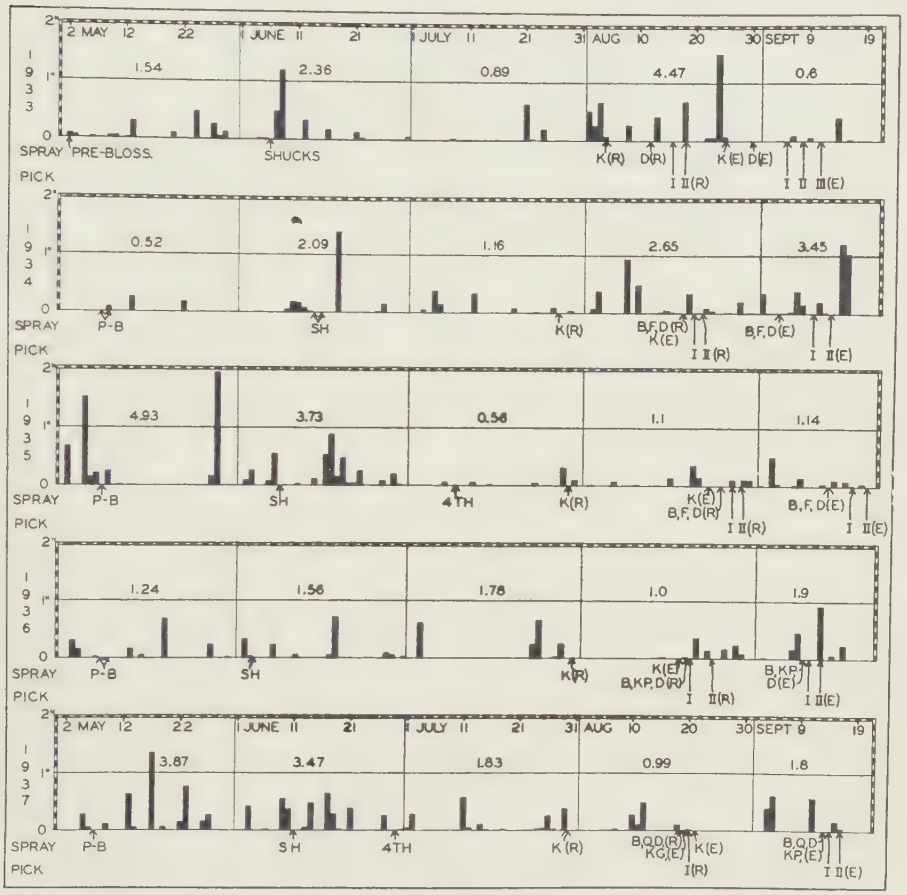

FIGURE 6. Distribution of rainfall and monthly precipitation during the growing seasons of 1933–37 inclusive, and dates of spraying and of harvest. P.B., pre-blossom spray; Sh., shucks spray, K and KG indicate the date of the last application of Koloform and Kolofog extra for the variety specified (applications of other materials were made in their respective plots at the same time). KP, B, Q, D indicate, for the variety specified, the date of last application of Kolopick, Bartlett's pre-pick, Qua-sul and dust respectively. (R) indicates the variety Rochester, and (E) the variety Elberta.

spot can be satisfactorily controlled in a journey of several thousand miles covered in a period of slightly over 2 weeks. In 1937, also, a commercial shipment of more than 50 boxes of sprayed Elberta peaches from the laboratory orchard was packed by a local concern and exported to various points in the British Isles. As a result of a questionnaire enclosed in each box, favourable reports were received concerning the absence of rot in this consignment.

DISCUSSION

Throughout the experiments recorded here, *Sclerotinia fructicola* has been the predominating cause of decay in harvested peaches. *Rhizopus nigricans*, however, appeared in many of the packs and caused considerable damage. *R. nigricans* differed from *S. fructicola* in that it spread more rapidly from peach to peach because of its more luxurious aerial growth. In this way it gave rise to "nests" of rotted fruit centred about the one in which infection first occurred. This habit would obviously tend to make *R. nigricans* a potentially more serious menace than *S. fructicola*. The latter was also capable of producing "nested" rots, but usually only in instances where the pack remained undisturbed for a longer interval than the 3 or 4 days between examinations. That *R. nigricans* remained of secondary importance was probably due to the fact that new baskets were used during picking and that, in grading, the fruit was not spread out on the packing house bench, but was either sorted from the basket or transferred to clean trays. Furthermore, the peaches were generally packed in new lugs, except in 1937, when the used lugs were first scrubbed and then sprayed with wettable sulphur before being returned to use.

Rots caused by other fungi, such as *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., etc., also attacked the fruit, frequently around the stem insertion, but only when the peaches were obviously near the end of their storage life and long since past the stage of edibility. Such rots were therefore considered as secondary and coincidental with a loss of resistance indicative of the initial stages of physiological breakdown. At the stage of development at which the secondary rots made their appearance, the skin of the peach ruptured very easily and could be "slipped" in a crumbling condition, due to the loss of cohesion between its constituent cells and between those of the skin and the flesh.

It is well known that with increasing ripeness, peaches become more susceptible to rot, a correlation apparent from the sigmoid character of the curves illustrating the increase in the amount of rot with the passage of time (Figure 5). From another point of view, this tendency is also reflected in the post-harvest behaviour of the disease in different lots, since it is indicated (Figurs 1 and 2) that, at given time within the period of observation, the incidence of rot was higher in the riper than in the greener peaches, both treated and checks. As already mentioned in connection with the 1935 experiments (Figure 4), there was considerable evidence that the first picks were not so subject to rot as the later ones. This not only seems to be another manifestation of the effect of the stage of maturity upon susceptibility, but also suggests that the maturation and ripening processes may take place more rapidly than the changes of colour would indicate. On the other hand, the differences between successive picks may also be explained on the basis that the maturity grades into which each pick was divided are arbitrary and may not be comparable with similar grades in other picks. The first hypothesis, however, has this to support it, that, in tests made with the aid of colour charts and a pressure tester, peaches on the tree were found to soften more rapidly than they changed colour. In effect, this means that, in a later pick, peaches at a given stage as denoted by ground colour would be softer than peaches of a similar colour in the preceding pick. This applies more particularly to the

"green" and "intermediate" grades of the experiments, as there was a reasonably close correlation between pressure and colour when the fruit was immature, or only just mature, and again when it was almost ripe.

Although there were some indications of increased decay as a result of careless handling (Figure 1 and 2), it was observed that bruises did not markedly contribute to the greater incidence of decay unless the skin of the fruit was broken at the same time. However, since the bruises themselves constitute a serious form of wastage and since there is no guarantee against breaking the skin, careful handling of peaches during picking and the later operations is imperative. It is also of interest in this connection that skin punctures of long standing, that were closed over at harvest time, were no more susceptible to rot than unbroken skin.

In consequence of spraying or dusting there was usually a marked reduction in the development of rot in peaches at various grades of ripeness (Figures 1 and 2) but it should be pointed out that none of the sprays or dusts, under the conditions of the experiment, gave absolute control. The chief importance of these preventive measures seems to lie in their delaying the onset of fungal wastage long enough to allow the sale and consumption of the fruit in a satisfactory condition. Moreover, the use of suitable fungicides, in combination with judicious post-harvest treatment in the matter both of handling and of carrying temperatures, permits the shipping and marketing of peaches picked ripe enough to develop to good quality and does away with the necessity, more or less imaginary, of shipping immature fruit.

Some differences were noted in the effectiveness of the fungicides used in these experiments (Figures 3, 4, 5). In this regard, the sulphur content of the spray mixture, the size of the sulphur particles, the time of application, the adhesiveness and, in the case of one of the materials, the solubility of the residue, each play their part. Generally speaking, the pre-harvest sprays, especially those with higher sulphur content, gave the best and most uniform results. Materials which, because of their tendency to mark the fruit, had to be applied 3 to 4 weeks before harvest, were almost as effective, though more variable, under most of the conditions encountered. This would suggest that such materials adhere well but may be less efficient than the pre-harvest sprays under critical conditions, because of the loss of coverage occasioned by the rapid growth of the peach fruit, during the last few days before harvest. Dust was, in many cases, as good as or even slightly better than the pre-harvest sprays, but in others much worse. This lack of uniformity and of reliability in the efficiency of the dust could be accounted for, in most instances, by the fact that the dust was more readily washed off by rain than were the sprays.

In those instances where the packs were transferred to low temperatures, 45° F. or lower, within 6 or 8 hours after picking, little or no rot appeared until after they were returned to higher temperatures. On the other hand, where they were held for an additional 24 hours before being cooled, the incidence of rot at the end of the period of low temperature was, as a general rule, about the same as that in lots not cooled at all. That is to say, the delay very often had the effect of nullifying the effects of cooling. Sometimes, in the 1935 shipment of Elberta to Saskatoon and in the 1937 experiments, for example, there were no differences between the lots cooled

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immediately after packing and those cooled 24 hours later. Whether or not the effect of the delayed cooling became apparent was evidently dependent on the conditions prevailing in the interval in question. The beneficial effects of prompt cooling, as indicated by Brooks and Cooley (4), are undoubtedly due to the fact that infection does not occur readily, if at all, at low temperatures, even though, once it has become established in the fruit, the fungus is capable of growing within the same range of low temperature. Since peaches take some time to cool in refrigerated cars (4), because the bulk of warm fruit is large in relation to the cooling capacity of the car, pre-cooling of peaches destined for long hauls is desirable, if facilities are available, and particularly if the weather has been unfavourable.

It should be observed that, during the 5 seasons covered by these investigations, conditions immediately preceding and following the harvest of the experimental material were not favourable for a serious outbreak of the disease, either in the orchard or in picked fruit. The control measures used in these experiments then, appear to be adequate for average seasons in Ontario. Under abnormal or more unfavourable circumstances, it becomes imperative not to overlook any precautions, either before or after harvest, if the fruit is to be marked without substantial loss.

RECOMMENDED CONTROL MEASURES

1. Practise strict sanitation in the orchard by cutting out blighted twigs at pruning time, or in early summer, and by removing mummied fruits from the tree after harvest. Mummies should be either gathered and burnt, or ploughed under.

2. Adopt a summer spray programme which should include a pre-blossom spray for the control of blossom blight, a "shucks" spray where necessary, for the control of scab and curculio, a spray or dust for brown rot, three or four weeks before harvest and again at harvest time, particularly if the weather is warm and damp at that time. An extra spray two or three weeks after shucks may be necessary in wet seasons, for brown rot of green fruits and mildew.

3. At harvest and during packing, handle the fruit carefully in order to prevent breaking of the skin or bruising. Finger bruises can be avoided by picking with a "cupped" hand instead of grasping the fruit with fingers and thumb.

4. Do not handle decaying peaches while picking for the market. If decayed fruits do find their way to the packing house, they should not be allowed to go through the grader or to contaminate the packing bench.

5. It is advisable to use clean utensils and containers during picking and packing operations. If the packing bench or the grader should become contaminated through contact with rotten peaches, they should be scrubbed with a strong solution of washing soda or a 10% solution of copper sulphate.

6. In preparing for long distance transportation, cool the fruit promptly after packing, preferably within 12 hours of picking. Do not cool first and pack afterwards because of the tendency of the chilled fruit to "sweat" on contact with warm air. In any case, low temperatures should be maintained until the shipment reaches its destination.

7. Precooling may not be necessary in dry seasons, but should be practised, if possible, when conditions at harvest are favourable to brown rot.

SUMMARY

1. In order to test the efficiency of various pre-harvest fungicidal treatments and the effects of other factors and conditions as related to the incidence of brown rot during long-distance shipping, numerous packs of peaches of the varieties Rochester and Elberta were shipped overland to the Canadian West and overseas to England, at which destinations they were examined upon arrival and at intervals thereafter. Duplicates of these shipments were given corresponding examinations at the St. Catharines laboratory.

2. While the riper grades of peaches were more susceptible to rot than the green, it was found possible to delay the incidence of rot in the riper grades long enough to permit marketing.

3. For either variety, the later picks were somewhat more susceptible to rot than were the first picks of the season.

4. A number of different forms of wettable sulphur were used in the experiments. In general, those sprays which could be applied immediately before harvest gave more uniform results than those which had to be applied 2 or 3 weeks earlier. Dusting with sulphur was also satisfactory except when rain intervened before harvest.

5. Wastage in the form of bruises and, to a lesser extent, of rots was reduced by careful handling during picking and packing operations.

6. The incidence of rot was usually much higher when peaches were cooled after a delay of 24 hours than when cooled shortly after picking.

7. In the case of shipments in which the car was iced only on departure there was little difference in the development of rot in treated and non-treated peaches.

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ROOT DEVELOPMENT IN RELATION TO ROOT ROTS OF CEREALS[1]

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INTRODUCTION

The interpretations presented in this paper are based largely on studies and observations made in Saskatchewan. Moreover the investigations have dealt chiefly with wheat.

Three distinct types of root rot are recognized in Saskatchewan at the present time. These are: (1) take-all caused by *Ophiobolus graminis* Sacc., (2) browning root rot caused by *Pythium* spp., following apparently nutritional disturbances and (3) common root rot caused by *Helminthosporium sativum* P. K. and B. and *Fusarium* spp. The first two diseases are important on wheat while common root rot attacks all cereals. In addition, a pre-maturity blight or wilt has been under observation by the workers at Saskatoon for a number of years and it has been grouped tentatively with the root rots. The cause of this malady is unknown. It is found in all cereal crops being quite common some seasons in oats.

Other types of root diseases quite probably occur but they have been overlooked so far. This is possible when the conspicuous symptoms are of short duration at the onset of the trouble, or when the symptoms are for the most part inconspicuous and uniform over large areas. The browning root rot may be considered a case in point, for once the critical seedling stage with its distinct symptoms has passed, diagnosis becomes difficult. Subsequent damage, however may be considerable, as a result of delayed maturity and weed competition.

In order, therefore, to understand fully root diseases, it is necessary not only to have some knowledge of root development throughout the season but also to keep the crop under observation from seed to harvest.

THE SEED INFLUENCE

The value of sound healthy seed is in general readily recognized but must be doubly stressed for areas where root rots are prevalent. The investigations of Mead (6) and others have shown that wheat seed injured in any way produces seedlings which are very susceptible to infection by root-rot fungi. Increased susceptibility is directly related to retarded or weak root growth associated with injured seed. When the young roots become established in the soil, they are entering a sphere of many hazards. These delicate organs must adapt themselves to an ever changing environment. Variations in soil moisture, temperature or salt content, to name a few factors, may be directly deleterious to root growth as well as influencing the attacks by parasitic fungi.

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[1] Contributio No. 565, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Read at the joint meeting of Canadian and American Phytopathological Societies, Ottawa, June 29, 1938.

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THE SEEDLING DEVELOPMENT

In Saskatchewan the seedling stage of wheat has been determined to persist for a period of about 6 weeks, by Simmonds and Sallans (13). It extends from the time of germination early in May to the second week in June when crown roots are becoming established. It is truly a critical period for the young plant. Wheat, however, is well adapted for the conditions prevailing in our principal grain growing areas. The soil temperature rarely exceeds 15° C. at the 6-inch level during this period but seldom retards root growth to any extent. The terminal bud remains at or slightly below soil level and so is protected against frost or other injuries which may affect the above ground parts. Consequently as the vital zones of the axis are well protected and injured leaves are soon replaced, root development goes on with little interruption.

It appears unlikely that the main environmental factors, such as temperature and moisture, interfere greatly with early root growth, except in isolated cases. At the end of the seedling period, therefore, the wheat plant will have about 5 seminal roots. Three of them the primary seminal root and the first 2 laterals will be well established in the soil, the primary reaching a depth of at least 1 foot. It has been established by Harris (3) that root growth of wheat is proportionately greater than shoot growth during the early stages. Root amputation tests on seedlings reported by Simmonds and Sallans (11) have shown that the seedling can withstand severe root injury. New roots are produced to replace those damaged. The total number of true seminal roots rarely exceeds 6. The injured seedlings survive if soil conditions are favourable, but do not fully recover. It was observed also that wounded seminal roots tended to encourage crown root growth. The same reaction occurred when the first roots became infected by *Ophiobolus graminis* according to Russell (8). Conversely, it is to be expected that any injury or inhibition of crown root growth might encourage the seminal roots. Krassovsky (5) has shown in water culture studies, that the removal of crown roots does stimulate the growth and activity of the seminal roots. Consequently dry soil conditions during the late seedling period may favour the progressive development of the first root system. However that may be, seminal root penetration is known to reach well into the sub-soil even with good crown root growth. With the appearance of tillers and crown roots, providing rains have been timely, the seminal roots need no longer play such an essential role. They remain functional, however, throughout the life of the plant. During the seedling stage they are the main source of support, and the severe injuries which parasitic fungi may cause are fully recognized.

Fungus infections of the seminal root system are rather common. Most of the cereal root rots can cause severe damage to seedlings and some probably inflict their greatest injury when the plants are young. The symptoms of browning root rot of wheat are most conspicuous early in June, when the lower leaves turn brownish and die, a behaviour indicating an impaired root system. A careful examination of the roots will reveal a heavy infection by *Pythium* spp., as the work of Simmonds, Russell and Sallans (14) and Vanterpool (16) has shown. The same or very similar shoot symptoms can be obtained by cutting off a portion of the numerous rootlets of the seminals. Moreover, newly formed crown roots frequently

show conspicuous *Pythium* lesions. Under good growing conditions, however, crown roots soon become established and the plants partially recover. The injury to seminal roots nevertheless results in a persistent impairment which is reflected by poor tillering and lateness in ripening. Amputations of the seminal roots similarly resulted in a reduction in the number of tillers and delayed maturity as shown by Simmonds and Sallans (12).

The take-all fungus, *Ophiobolus graminis*, commonly attacks the seminal roots and may cause severe injury. When they are completely destroyed, as they occasionally are, the plant can only survive by receiving its nourishment from the crown roots (14). Generally speaking the critical period of infection by *O. graminis* appears to come later as the fungus advances upward to the subcrown internode and crown. This phase of the attack will be discussed later.

The common root-rot fungi, *Helminthosporium sativum* and *Fusarium* spp., may at times cause considerable damage to seedling roots. The amount of injury fluctuates, for the pathogenicity of these fungi is greatly influenced by environmental conditions. If the pathogens are carried on the seed or are active in the soil at seeding time, the young roots and shoots may be invaded and the seedling destroyed. However, complete destruction of seedlings before they have emerged is not, so far as we know at present, of frequent occurrence in Western Canada. The prevalence of these fungi, nevertheless, makes it very probable that the roots of all cereal crops bear lesions, in varying degrees, throughout the season. The extent to which the lesions spread and cause damage is largely dependent upon the soil temperature. The natural rise in soil temperature, as the season advances, will tend to encourage any incipient root infections until there occurs what might be termed the second critical phase. The latter phase involves consideration of the disease when it has developed on the subcrown internode and crown.

THE POST-SEEDLING DEVELOPMENT

It is generally recognized that there is a strong correlation, in most wheat varieties, between the number of tillers and the number of crown roots. These surface roots, however, do not grow to any extent or increase greatly in number under the usual crop conditions in the semi-arid regions of Western Canada (13) because competition for surface moisture is too severe. This fact is particularly true when rainfall is below normal and must be taken into consideration in the interpretation of root disease reactions.

Recent work by Simmonds and Ledingham (15) has shown that fungi are relatively more abundant in the top soil layers. It is also in the same zone that the vital parts of the plant's axis, *viz.*, the crown, the subcrown internode, and the seminal root juncture at the first node, develop. Any injury, even quite severe, to a distal portion of the seminal system may be repaired by replacement with little damage or shock. A severe lesion, however, at the subcrown internode or seminal root juncture will cut off the entire lower root system, with distinctly deleterious results. It is readily realized, therefore, that conditions which inhibit crown root growth, predispose the plant to great hazards. The increase in soil temperature with

the advance of the season will encourage the development of the common root rot fungi, *H. sativum* and *Fusarium* spp. It is believed that they cause considerably more damage than is generally recognized. Sallans (9) has shown that severely infected subcrown internodes may reduce the yield of wheat plants 30 to 45 per cent. The same fungi invade the crown roots as well as the tiller buds and tillers. Infections at the crown are frequently responsible for the blighting of tillers so noticeable some seasons in durum wheats.

The take-all fungus, *O. graminis*, as mentioned above, commonly advances into the region of the crown from infected seminal roots. It rapidly invades the crown and adjacent tissues. The virulence of the infections is easily recognized by the premature bleaching of the plant followed by death. The previous destruction of the seminal roots which frequently happens, merely hastens its final collapse.

It is evident, therefore, that the extent of injury from post-seedling infections depends largely upon the condition of the seminal root system. A plant handicapped by an impaired primary root system is bound to suffer permanent damage, if not death, when conditions are favourable for an invasion of the crown or subcrown zones. The remarkable power of the wheat plant to repair the damage caused by crown root amputations, was well shown in the studies conducted by Simmonds and Sallans (12). Favoured by timely rains, it appears that only the most virulent root and crown invasions can completely destroy a wheat plant.

GENERAL OBSERVATIONS

It is well known that cereals can withstand rather severe drouth. Wheat is well adapted to semi-arid conditions. Once it is well established it can survive and reach maturity with a minimum of surface moisture. The rapidly penetrating seminal roots, taking advantage of the usual spring rains, soon reach and tap any moisture at the lower levels. These roots may penetrate to a depth of 4 feet or beyond. A complete crop failure is to be expected only when the sub-soil moisture is depleted. Absorption takes place from all levels, to the maximum depth of penetration according to the work of Weaver, Jean and Crist (17) with barley, rye and other crops.

Wheat plants restricted to seminal roots only are inclined, of course, to show a reduction in yield. Observations have shown that this is true in dry years. But what is of greater interest and to some extent a compensating factor, is the high quality of such wheat. Conversely, years of abundant rainfall and high yield, when extensive crown root development takes place, almost invariably results in much wheat of low quality as reported by Shutt (10) and Hopkins (4). It is possible that there is a physiological distinction in function between crown roots and seminal roots as was indicated in Krassovsky's (5) experiments. The different feeding levels, in part at least, for the 2 systems may have some influence in this connection. Gericke's investigations (1, 2), show that there is a distinct relationship between vegetative growth, including tillering, and the protein content of the wheat grain. Protein content is associated with the absorption of nitrogen while the "quality of protein" in turn is influenced by variations in mineral nutrition. Neatby and McCalla (7) found in their

plant breeding studies, when considering genetical relations chiefly, that there was an association between yield and protein content in wheat and barley. When yield was increased the protein content decreased with few exceptions. Data on root development would have been of value in material of this kind. It is interesting to speculate on the possibility of breeding a wheat plant supported only by the seminal system. Such a wheat would be somewhat drouth resistant because of the deeply penetrating roots. The restricted vegetative growth of one or two culms, would, we are led to believe, produce wheat of high quality. The decrease in yield could be partly compensated for by an increase in seeding rate. It is suspected that segregates having weak or no tillering ability, are usually discarded on account of low yield in most plant breeding projects.

In regard to the pathological significance of such a development, the situation may be viewed with hope from at least one angle. Some wheat varieties appear to be more resistant than others to subcrown internode infections. If a varietal character of this type is established by future tests, it should be of the greatest significance. A healthy zone of such pivotal importance as the crown, subcrown internode, and the seminal root juncture, is vital for the normal functions of the main axis; it is only of slightly less importance to good tiller development.

There are many other interesting angles to investigations dealing with the pathology of cereal roots. These cannot be enlarged upon here. The ever changing soil medium in which the roots must live presents many multilateral problems. The ability of the root system to meet by modifications many unusual circumstances, such as is so evident in pot cultures, expresses well the great adaptability of these organs. On the other hand, we have to recognize their sensitivity to abnormal conditions including disease.

SUMMARY

Interpretations on root disease reactions, in relation to root growth, were based largely upon experience gained in studying these diseases in Saskatchewan.

It was pointed out that the state of health of cereal seeds is of prime importance. Seeds weakened in any way invariably produce sickly plants predisposed to attacks by root rot fungi.

The seedling stage, especially in wheat, was considered as that period from germination until crown roots emerge. During this stage the plants are supported entirely by the seminal roots. Root infections during the seedling period are shown to be responsible for much injury, usually accompanied by distinct shoot symptoms.

Infections of the crown, crown roots, and tiller buds were considered in a discussion of post-seedling invasion. The damage caused through the latter stage of disease development is particularly severe when the seminal roots are also infected.

The two root systems of wheat, the seminal and crown, were shown to play important rôles in the growth of the plant. The seminal roots penetrate deeply, reaching sub-soil moisture early in the season. They remain functional throughout the life of the plant. The crown roots support any

increase in growth when tillers develop. Root rot infections are compared with artificial injuries to both root systems. The reactions are comparable in many respects.

Some observations were made on the possibilities of developing a wheat plant supported only by the seminal roots. The suggestion was considered both from the physiological and pathological angles.

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SHRIVELLING OF WHEAT KERNELS BY STEM RUST AND ITS EFFECT ON SEED VALUE[1]

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The value of shrivelled wheat as seed has been a much debated topic following severe rust epidemics, such as occurred in 1904, 1916, 1927 and 1935. Bolley (1) conducted some tests in North Dakota in an effort to determine the seed value of shrivelled wheat from the 1904 crop. Bracken (2), in Saskatchewan, conducted greenhouse tests on seed from the 1916 crop. The latter found that shrivelled wheat germinated well, but the seedlings were not vigorous. Bridge (3), working with seed grown in 1916, also found that the sprouts from shrivelled grains were weak, the weakness being in proportion to the amount of shrivelling. He considered that the use of shrivelled grain delayed the time of ripening of the crop. Kiesselbach and Helm (5) studied the relation of the size of the seed and sprout value to the yield of small grain crops. As an average for all tests during 2 years 1913 and 1914, the ratios for the moisture-free weight of unselected seed to that of large and small seed were respectively 100 : 127 and 100 : 85, while the ratios for the total sprout value were 100 : 123 and 100 : 88 respectively. In a 2-year yield test of unselected, large, and small seeds, of 2 winter wheat varieties, the average relative seed weights were 100, 134.6 and 86.9, with corresponding sprout values of 100, 133 and 92.3. This indicates a rather close relationship between the size of seed and its sprout value. They found also, that though the small or shrivelled seed of winter and spring wheat weighed 66% as much as large seed and had a sprout value 68% as great, the laboratory germination of the 2 grades was practically equal. Field tests showed that when small and large seeds were planted in equal numbers, the small seed yielded 11% less when planted alone and 24% less when in competition with large seed. If equal weights of large and small seed were sown at a rate optimum for the large, the yields were alike; if equal volumes were sown, as with a drill set at a uniform rate, slightly smaller yields resulted from the small seed, though the difference was so small as to have little practical significance. In addition, they found that at the unusual seeding depths of 4 and 6 inches, the germination of small seed of spring wheat was 87.1% and 92.1% respectively, as compared with 100% for large seed. The yield of grain also was greatly reduced being 63.2 and 62.8% of the yield from large seed sown at 4 and 6 inches. Stoa, Brentzel and Higgins (9) found that plants from shrivelled seed from the 1935 crop were smaller and less thrifty than plants from plump seed. They concluded that a stronger germ and a greater reserve food supply in plump seed results in more vigorous seedling growth. In their greenhouse experiments, no significant correlation existed between plumpness of seed and germination, but there was a decided positive correlation between plumpness of the seed and the size and vigour of the young plants. They studied the effects of unfavourable conditions on seedlings and found that the small plants from shrivelled seeds did not

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have the same ability to recover from wilting due to drought as did the stronger plants from plumper seed. They found also, that the loss of seedlings due to a killing frost followed by drought or high evaporation was greatest when shrivelled seed was sown. Taylor and McCall (10) separated the small and shrivelled seed from spring and winter wheat and grew it along with plump seed in the greenhouse. They found that the plants from large seed had more seminal roots than those from small seed, the difference being 5.7% for fall wheat and 11.4% for spring wheat. Mead (7) has shown that the stand and yield from shrivelled wheat is less than that from normal wheat in the presence of the root-rot fungus *Helminthosporium sativum* P. K. & B. Simmonds and Scott (8) referred to Kiessling's (6) determination that formaldehyde, in ordinary seed treatment dosage caused injury to seed grain, and in their own experimental work they found that ordinary formalin treatment injured wheat seeds and gave poor protection against fungi which cause root-rot.

In 1935 the wheat crop of Saskatchewan was severely damaged by stem rust. The widespread occurrence of the disease, and the severity of its attack necessitated the use of a large amount of shrivelled wheat for seed in 1936. Greenhouse investigations into the seed value of this shrivelled wheat were conducted by the Dominion Plant Pathological Laboratories at Saskatoon and Winnipeg, the Saskatchewan Department of Agriculture at Regina and the Field Husbandry Department at the University of Saskatchewan. The results of the investigations were compared and the committee's report advised those who were forced to use shrivelled seed to clean it heavily to remove the lightest seeds and to sow at the usual rate but as shallow as moisture conditions permitted. The Field Crops Branch of the Saskatchewan Department of Agriculture conducted a survey of the 1936 crop grown from shrivelled seed (11). The results of the survey showed that for the most part, spring conditions were good, while the summer was hot and dry, and that in the areas of light rainfall, and also where conditions were favourable, shrivelled wheat produced quite satisfactory stands and yields. Field observations made during the survey showed no difference in crops from plump and shrivelled seed, and the yield from No. 1 Northern seed was not significantly greater than that from the lower grades of shrivelled wheat. These results are interesting in the light of greenhouse tests described in this paper, which show that the early vigour of seedlings from rusted wheat was considerably lower than that of seedlings from normal seed.

MATERIALS AND METHODS

The seed which was used included samples of the commercial grades of Marquis, Nos. 4, 5, and 6 Special and Feed (rusted), all grown in the same district. A sample of No. 1 Northern, grown in a rust-free area, was used as a check. All of this seed, from which broken kernels were removed, was grown in soil, in sand, and on blotters in the greenhouse and in small plots in the field.

GREENHOUSE AND LABORATORY TESTS

Shrivelling of Wheat Kernels due to Stem Rust

Portions of one sample of each of No. 1 Northern and Feed wheat were soaked in water overnight and the embryos were dissected from 500

kernels of each kind, in 100-kernel lots. Dr. R. K. Larmour, of the Department of Chemistry, University of Saskatchewan, made an analysis showing the relative amounts of embryo, fibre, endosperm and moisture in these dissected kernels. The results of the analysis are shown in Table I.

TABLE 1.—A COMPARISON OF THE AMOUNTS OF EMBRYO, FIBRE, ENDOSPERM AND MOISTURE IN NORMAL AND SHRIVELLED KERNELS OF WHEAT*

Seed	Tissue	On moisture-free basis			Moisture in fibre and endosperm
		Weight	Proportion of normal	Proportion of whole kernel	
		grams	%	%	%
Normal	Embryo	.1019		3.41	
	Fibre	.0977		3.27	3.15
	Endosperm	2.7873		93.32	
Shrivelled	Embryo	.0424	41.6	3.73	
	Fibre	.0558	57.1	4.94	4.51
	Endosperm	1.0329	37.0	91.31	

* The results are the averages of five determinations for each type of seed.

These dry weight determinations, based on one sample of normal wheat, and one sample of shrivelled wheat indicate that all parts of the kernels which were produced on rusted culms suffered a reduction in weight. The dry weight of the embryos was 41.6% of the dry weight of the embryos of normal kernels, that of the fibre 57.1% of normal fibre and that of the endosperm 37.0% of normal endosperm. Thus, in this shrivelled sample the endosperm tended to shrink more than the embryo or fibre, and it constituted a smaller proportion of the bulk of the kernel than it did in the normal wheat. A comparison of the dry weights of the individual lots of embryo tissue was made with the weights of the corresponding lots of endosperm tissue and with whole kernel weights also. This comparison showed that the embryos of shrivelled seeds were proportionately larger in the shrivelled kernels than in the normal kernels. The differences were statistically significant. The data are given in Table 2.

TABLE 2.—THE RELATION OF THE WEIGHT OF THE EMBRYO TO THAT OF THE ENDOSPERM AND OF THE WHOLE KERNEL IN NORMAL AND SHRIVELLED (RUSTED) WHEAT

Lot No.	Embryo weight expressed as percentage of weight of			
	Endosperm		Whole kernel	
	Normal	Shrivelled	Normal	Shrivelled
	%	%	%	%
1	3.70	4.11	3.46	3.75
2	3.62	3.80	3.38	3.48
3	3.61	4.18	3.37	3.82
4	3.70	4.27	3.45	3.90
5	3.63	4.15	3.38	3.79
Average	3.65	4.10	3.41	3.75
Diff.	.45		.34	
S	.1304		.1170	
t	5.45		4.59	

5% point, 2.306; 1% point, 3.355.

The relative size of the embryo and endosperm in normal and shrivelled kernels is shown in Figure 1 which is a photograph of longitudinal and transverse sections of representative kernels. In addition to the severe reduction in the endosperm of shrivelled seeds, there is a great increase in the depth and width of the groove. This is important in relation to seed-borne fungi, particularly those causing root rot.

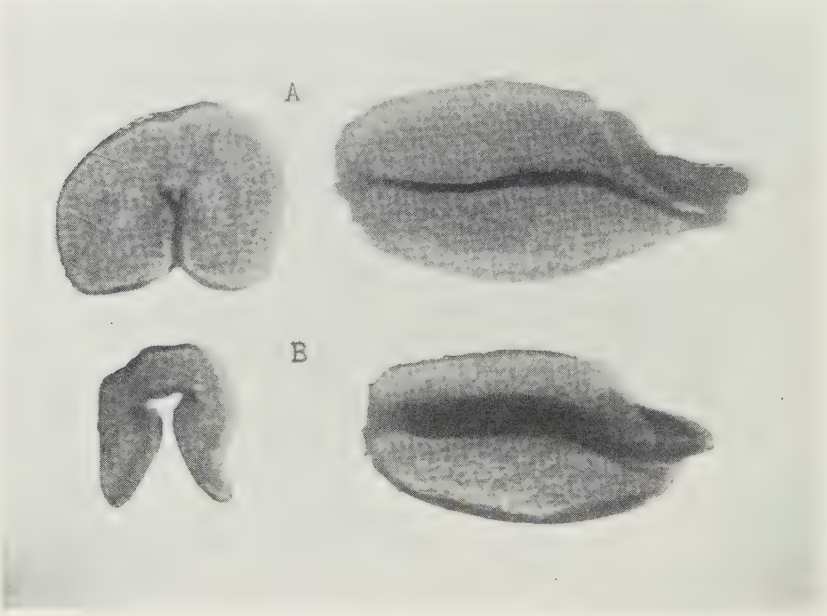

FIGURE 1. A photograph of transverse and longitudinal sections of wheat kernels. A. normal. B. shrivelled (rusted). The transverse sections were cut near the embryo; the longitudinal sections were cut almost vertically through the groove.

Seed Content and Germination of the Samples

The samples were compared with respect to their weight, and their germinability. Because of the light weight of the shrivelled kernels and their good germination, the low grade samples contained many more viable kernels than the high grade samples. Three hundred seeds of each sample

TABLE 3.—THE WEIGHT, SEED CONTENT, AND GERMINATION OF COMMERCIAL GRADES OF WHEAT SHOWING VARIOUS DEGREES OF SHRIVELLING

Commercial grade	Weight per measured bushel	Weight per thousand kernels	No. kernels per bushel	Germination	No. germinable kernels per bushel
	pounds	grams	thousands	per cent	thousands
No. 1 Northern	63.5	23.9	1205	99.3	1196
No. 4 Special	54.0	16.5	1484	97.0	1439
No. 5 Special	52.0	15.1	1560	95.0	1483
No. 6 Special	50.0	15.0	1511	98.7	1492
Feed	48.0	11.8	1845	94.3	1739

were grown in soil in the greenhouse. The germination of the samples is given in Table 3 along with data on weight and seed content.

These results show that the shrivelled wheat germinated well, though not as well as normal wheat and that there were more germinable kernels per bushel in the low grade samples. An attempt was made to improve the low grade samples by screening them and removing some of the kernels which were most shrivelled. The samples were shaken on a No. 7 zinc screen with round holes, and 20% by weight of number 4 Special, 30% of number 5 Special, 40% of number 6 Special and 50% of Feed were removed. The removal of the small kernels increased the weight of the various grades, improved their germination slightly, and reduced the number of germinable kernels per bushel.

Early Vigour of Shrivelled and Plump Wheat.

While the shrivelled kernels germinated in almost as great percentages as plump kernels, the seedlings which they produced were not all as vigorous as those from the normal seed. This was shown by planting 200 kernels from each sample in sand. When the seedlings were 4 days old, they were removed from the sand and were classified as *strong*—with 3 or more healthy roots and a plumule; *fair*—with 1 or 2 roots and a plumule; and *poor*—with no roots, but having a plumule. The results are shown in Table 4.

TABLE 4.—THE COMPARATIVE VIGOUR OF SEEDLINGS PRODUCED BY 5 COMMERCIAL GRADES OF WHEAT WHEN GROWN IN SAND IN THE GREENHOUSE FOR 4 DAYS AT 20° C.

Commercial grade	Seedling classes			Non-germination
	Strong	Fair	Poor	
	%	%	%	%
No. 1 Northern	93.5	1.5	2.5	2.5
No. 4 Special	91.0	5.5	2.0	1.5
No. 5 Special	79.5	7.5	9.5	3.5
No. 6 Special	85.0	6.0	6.0	3.0
Feed	85.0	7.5	2.5	5.0

These results show that shrivelled seed produced more weak seedlings than plump seed. This is significant in view of the fact that unpublished researches have shown that the mortality among the weak seedlings is greater than among the strong ones.

Emergence in Greenhouse Tests

The samples of normal and shrivelled wheat were sown in soil in the greenhouse, the seeds being sown 1.5 inches deep in sandy loam soil. Four hundred seeds of each sample were planted. The moisture in the soil in one-half of the crocks was maintained at about 30% of its moisture holding capacity and in the other half it was kept at about 60%. There was no particular difference in the total emergence when the soil was relatively dry and when it was moist. The total emergence for the moist lots was 378, and for the dry, 375. In two samples the emergence was better in moist than in dry soil, in one sample it was the same and in two samples

it was better in dry soil. Thus soil moisture, in excess of the minimum for germination, was not a limiting factor in the emergence of these samples. The emergence of No. 1 Northern wheat was better than that of the shrivelled samples. Emergence data are given in Table 5.

Root and Shoot Development

In these experiments, the seed was sown in soil in the same way as in the emergence tests, and in washed, sterilized sand as well. After various periods of growth, the plants were washed free of soil and sand, and measurements were made of the lengths of the shoots in the soil cultures, and the lengths of the shoots and roots in the sand cultures. Dry weights of the shoots were recorded also. The data are represented in Figure 2.

TABLE 5.—THE EMERGENCE OF NORMAL AND SHRIVELLED WHEAT IN SOIL IN THE GREENHOUSE

Commercial grade	Emergence	
	In moist soil	In dry soil
	%	%
No. 1 Northern	90	86
No. 4 Special	70	72
No. 5 Special	64	64
No. 6 Special	85	78
Feed	69	75

The results of the experiments show that under greenhouse conditions, top growth from No. 1 Northern seed was greater than that from low grade seed at all stages. There was a greater difference between the various grades of seed in the dry weights of the seedlings which they produced than in their heights, the reason being that the young seedlings from low grade seed were rather spindly. Important also, is the reduction in the root systems produced by the shrivelled samples. Unpublished researches showed a marked positive correlation between top and root development in the seedling stage of wheat. All of these data indicate that the vigour of early growth of wheat seedlings depends primarily upon the condition of the seed from which they arise. Weak seedlings probably indicate weak seed.

Treatment with Formalin.

Since formalin is still used extensively as a seed treatment against bunt or stinking smut of wheat, and since it has been shown by many workers to be the cause of reduced germination, tests were conducted to determine its efficacy when used on shrivelled wheat. In laboratory tests conducted in co-operation with the Saskatoon branch of Production Service and Plant Products, a progressive decrease in germination of formalin-treated seed was found as the lower grades were reached. The treatment did not reduce the germination of No. 1 seed, but it lowered that of No. 4 Special 9%, No. 6 Special 14%, and Feed 16%. The speed of emergence also was affected adversely by treatment with formalin, the effect being greatest in the lower grades. It is generally considered that the greater the speed of its emergence, under normal germinating conditions, the greater is the value of the seed.

The Effect of Freezing and Cutting the Seedlings

Seedlings from the various samples grown in the greenhouse to the fourth-leaf stage, were exposed to freezing temperatures on 2 successive days. On each day the temperature dropped to 21° F. during the 4-hour

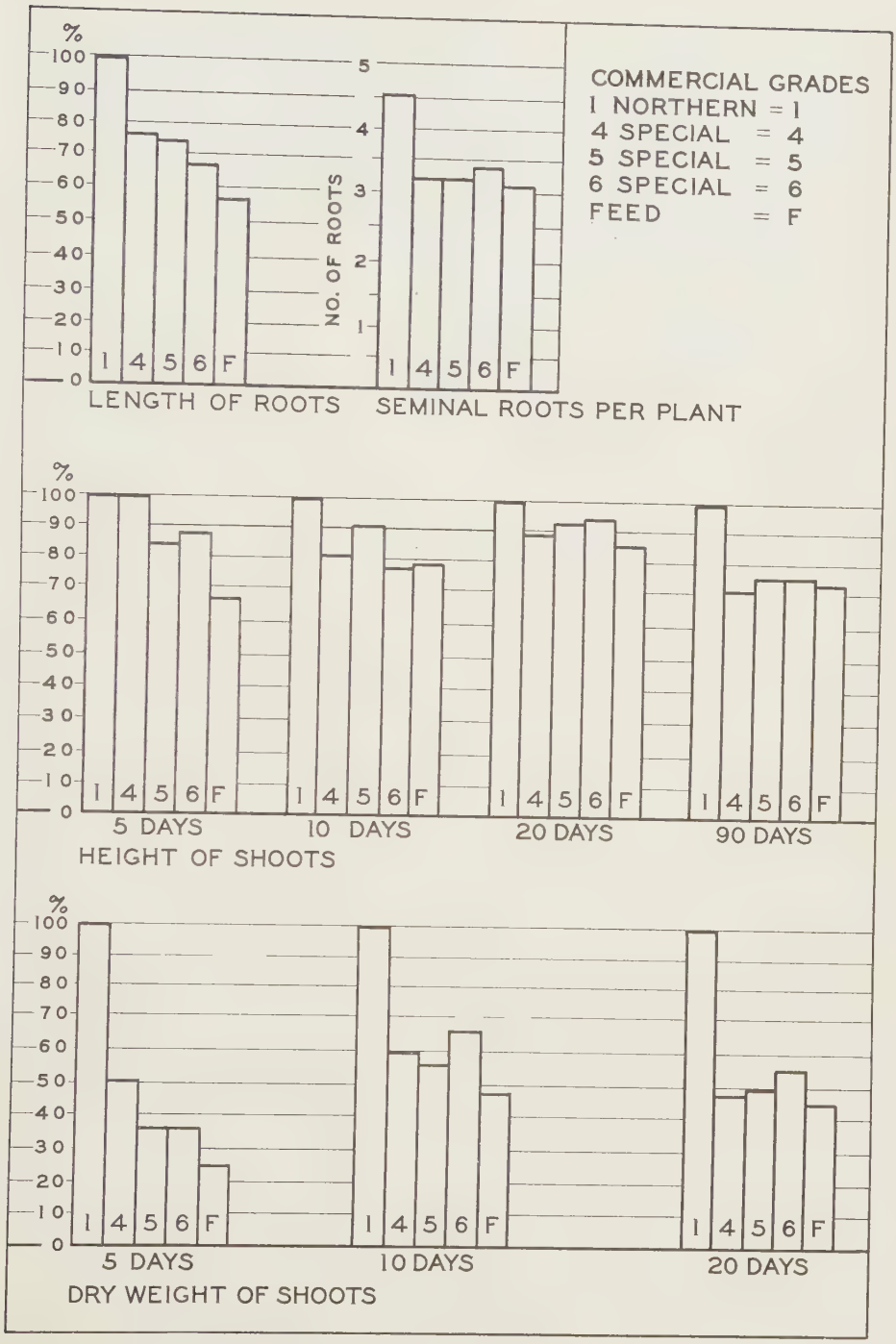

FIGURE 2.—Root and shoot development of normal and shrivelled wheat in the greenhouse. The plants from which root measurements were taken were grown in sand; those used for measurement of the shoots grown in soil.

exposure. The seedlings were then removed to a moderately warm green house and were exposed to strong artificial light overnight. The more vigorous seedlings from plump seed were observed to stiffen first during the period of frost but on the following morning, by actual count, the percentage of injured leaves was approximately the same in all samples. Although measurements taken over a period of 30 days showed that the frost had caused stunting of all of the shoots, this was much greater in the seedlings from No. 1 Northern wheat than in the others, probably on account of the greater vegetative development of normal seedlings and consequently greater susceptibility to frost.

The ability of seedlings from each of the samples to recover after severe injury such as hail or severe soil drifting would cause was tested. Ten plants from each sample were cut off at the ground line when they were five weeks old. The same number were grown as controls. After a month's growth, the height of all of the tillers of the cut and control seedlings was measured. The averages of these measurements are given in Table 6.

TABLE 6.—COMPARATIVE TOP DEVELOPMENT OF CUT AND CONTROL SEEDLINGS FROM 5 COMMERCIAL GRADES OF WHEAT, EXPRESSED IN CENTIMETERS AND AS PERCENTAGES OF THEIR RESPECTIVE CONTROLS

	No. 1 Northern		No. 4 Special		No. 5 Special		No. 6 Special		Feed	
	cm.	%	cm.	%	cm.	%	cm.	%	cm.	%
Cut	44.0	56.4	24.2	42.5	29.4	51.9	20.3	35.1	23.8	41.9
Control	78.0		57.0		56.7		57.9		56.7	

Standard error of a difference 6.47 cm.

An analysis of variance of the individual measurements was made by the method outlined by Goulden (4). This analysis showed that the differences between the heights of cut and control seedlings of all samples were very significant. The difference between the height of cut seedlings from No. 1 Northern seed and that of the cut plants from any of the shrivelled samples was very significant also. The ratios of the heights of cut and control seedlings from the 5 samples, shown as percentages in Table 6 indicate that the seedlings from the shrivelled samples were less able to recover from severe damage than those from normal seed.

Reaction to Drought

A number of seedlings from each grade of wheat were allowed to grow under good moisture conditions for 2 weeks and then were given no water for 7 weeks. Similar plants were watered regularly and served as controls. Wilting was evident in all of the unwatered seedlings during the fourth week of drought conditions, and it was seen in the largest seedlings from No. 1 seed first. At the end of the period, data were recorded showing the effect of lack of water on height and dry weight. These data are shown in Table 7.

TABLE 7.—THE EFFECT OF LACK OF WATER FOR 7 WEEKS ON WHEAT SEEDLINGS GROWN IN THE GREENHOUSE. THE SEEDLINGS WERE 2 WEEKS OLD WHEN THE DRY PERIOD BEGAN

Commercial grade	Reduction in height	Reduction in dry weight	Condition of seedlings at end of dry period
	%	%	
No. 1 Northern	62.1	61.5	20% dead, others wilted and terminal leaves dead.
No. 4 Special	49.2	62.9	All living; all partly wilted, erect, "staring".
No. 5 Special	39.3	62.4	All living; all partly wilted but less than No. 1.
No. 6 Special	33.2	45.1	All living; all about one-half wilted; better colour than 1, 4 and 5.
Feed	41.7	59.2	All living; all very slightly wilted.

Lack of water reduced the height of the seedlings from No. 1 Northern seed more than that of the smaller plants from shrivelled seed, but the reduction in dry matter was, with one exception, much the same in all of the samples. The seedlings from No. 1 Northern seed being much larger and more vigorous, probably used up the available water supply more quickly than did the less vigorous plants from the other samples. They would then be less able to withstand the drought period than the plants from shrivelled seed. When the surviving seedlings of the 5 samples were given water, they all recovered, and those from No. 1 Northern seed regained their turgid condition first. Heading in all of the dried plants occurred much later than in the controls.

FIELD TESTS

Resistance to Common Root-rot

Portions of the samples of No. 1 Northern and No. 5 Special wheat were inoculated with a spore suspension of *Helminthosporium sativum* P. K. & B. and were sown along with uninoculated seed in small plots in the field. Considerable pre-emergence blight occurred in the plots of both plump and shrivelled inoculated wheat, but fewer plants were able to survive from the shrivelled seed than from the normal seed and consequently the stand and yield from the former was poorest. This reduction in emergence and yield varied directly with the amount of shrivelling, being greatest in the lowest grades. Table 8 is a short summary of the results obtained.

TABLE 8.—THE EFFECT OF COMMON ROOT-ROT ON EMERGENCE AND YIELD OF NORMAL AND SHRIVELLED WHEAT IN THE FIELD

Seed	Reduction in emergence	Reduction in yield
	%	%
Normal	11.0	7.0
Shrivelled	40.0	40.0

Seed Treatment and Shrivelled Seed

Laboratory tests showed that the germination of shrivelled wheat was seriously diminished by treatment with formalin. Field tests were planned to compare the efficacy of formalin and organic mercurial dusts when used on shrivelled seed in the field. Some No. 1 Northern wheat and some No. 5

Special were soaked in a 1 : 320 solution of formalin for 30 minutes, drained and covered for 2 hours, and then spread out to dry. Similar quantities of the 2 samples were treated with New Improved Ceresan at the rate of one-half ounce per bushel, and with Leytosan at the rate of one ounce per bushel. This treated seed was sown, along with untreated seed from the same samples, in five-row replicated plots, one rod long. The data from these plots supported the laboratory test in showing that formalin causes considerable injury to shrivelled seed. The difference in emergence between shrivelled wheat treated with formalin and the same wheat treated with New Improved Ceresan or Leytosan was highly significant statistically. The same is true of the yield of threshed grain. The stand and yield of No. 1 Northern wheat also were reduced by treatment of the seed with formalin but the reduction was less than that of shrivelled seed. No. 1 seed treated with the organic mercurial dusts had a significantly greater emergence and slightly greater yield than that treated with formalin. The yield from No. 1 seed treated with New Improved Ceresan and with Leytosan was less than the yield from untreated seed but the differences were not statistically significant. Emergence and yield data are given in Table 9.

TABLE 9.—A COMPARISON OF THE EFFECT OF SEED TREATMENT WITH FORMALIN, NEW IMPROVED CERESAN AND LEYTOSAN ON THE EMERGENCE AND YIELD OF NORMAL AND SHRIVELLED WHEAT, GROWN IN THE FIELD*

Seed	Treatment	Emergence	Yield
		%	%
Normal	Untreated	100	100
	Formalin	69	80
	Ceresan	89	87
	Leytosan	87	91
Shrivelled	Untreated	100	100
	Formalin	55	78
	Ceresan	104	103
	Leytosan	105	100

*The emergence and yield of treated seed are recorded as percentages of the respective emergence and yield of untreated seed.

Yield from Untreated Seed

In this test, normal and shrivelled wheat which had not been treated in any way were sown in 5-row plots, 1 rod long, replicated 4 times. Seeding was done with a hand seeder set to sow about 250 seeds of plump wheat per rod. Because of the shrivelled condition of the kernels in low grade samples, many more kernels of No. 4 Special, No. 5 Special, No. 6 Special and Feed, than of No. 1 Northern would be sown. The plots sown with shrivelled wheat had a thicker stand of plants than those in which normal wheat was sown. However, the more vigorous plants from No. 1 Northern seed produced the greatest number of productive tillers per plant. The yields from the 3 inner rows of each plot were obtained and are summarized in Table 10.

The yields from individual plots of the 5 grades of wheat were analyzed statistically, and only the difference between the yields from No. 1 Northern and No. 4 Special was found to be significant.

DISCUSSION

The results given in the literature which has been reviewed and the data presented in this paper agree in many details. Previous findings

TABLE 10.—THE YIELDS FROM 5 COMMERCIAL GRADES OF WHEAT, SOWN IN REPLICATED PLOTS

Commercial grade	Yield		Weight per bushel
	Bushels per acre	per cent*	
No. 1 Northern	16.16	100.0	62.5
No. 4 Special	14.20	87.3	62.0
No. 5 Special	15.63	96.1	62.5
No. 6 Special	14.61	89.8	62.0
Feed	14.34	88.2	63.0

*The yield from No. 1 Northern is taken to be 100 per cent.

have shown that wheat which had been shrivelled by stem rust germinated well, but that the resulting seedlings were weak, as compared to plants from normal seed. This weakness took the form of stunting, spindliness and general lack of thrift. The investigators concluded that this weakness was caused by injury to the embryo and reduction in food supply during the maturation of the shrivelled seed. The present data taken from the greenhouse tests show that shrivelled kernels germinated well but produced weak seedlings and that these seedlings grew more slowly and contained less dry matter than normal seedlings. This was true of the early growth, particularly, when the reserve food in the seed was being utilized by the young plant. One of the experiments showed that there actually was less reserve food, in the form of endosperm tissue, in shrivelled kernels than in normal kernels. This reduction in the weight and size of the endosperm was accompanied by a smaller reduction in the embryo.

The general lack of thrift in seedlings from shrivelled seed is probably due to the under development of the embryo in the seed (see comparative embryo weights, Table 1), and to the inadequate supply of carbohydrates in the endosperm. The weaker seedlings from shrivelled seed also are unable to grow as rapidly as those from the plump seed due to reduced capacity to synthesize carbohydrates, resulting from smaller leaf areas and less extensive root system. The results of experiments described in this paper confirm previous findings in showing that the root systems of plants from shrivelled seed are reduced. This retardation in root development is very important when one considers its relation to root rot.

The present data do not agree entirely with those of other investigators in relation to the resistance of seedlings from shrivelled wheat to drought and frost. In the present experiments it was found that in the greenhouse, these seedlings did not suffer as much stunting after being frozen as normal seedlings and the difference was thought to be due to the greater vegetative growth of the latter. The effect of prolonged drought was found in the present experiments to be about the same on both classes of seedlings. Other authors found that plants from shrivelled seed were less able to recover from frost and drought than plants from normal seed.

In the present experiments there was a significant difference in the effect of cutting normal and weak seedlings, the injury being greatest on the latter. Many of the conditions which prevail during the early development of wheat in Saskatchewan approach this type of injury in severity. Two of these conditions are soil drifting and insect injury.

In a previous presentation (7) from this laboratory data were presented which showed that the resistance of wheat seedlings grown from shrivelled seed, to a root-rot organism *Helminthosporium sativum*, was less than that of normal seedlings. The present experiments, also conducted in the field, support these findings. It is suggested that part of the difference in the resistance of weak and normal seedlings lies in the possession by the latter of a greater potentiality of growth in the form of more tiller buds and more conductive tissue.

Our findings with respect to seed treatment with formalin are in agreement with the results obtained by other workers. Formalin injures all grades of wheat seed, the amount of injury being related to the quality of the seed. It was found that as the lower grades of shrivelled wheat were reached, the injury to germination increased. The present experiments also show that it is advisable to substitute an organic mercury dust for formalin, when shrivelled seed is being treated.

It will have been observed that greenhouse tests described here and by other investigators indicated that wheat seedlings from seed shrivelled by stem rust are weak in most respects. It has been assumed by some that those seedlings would be weak in the field, and would produce a late crop and low yield. The results of field tests do not support this view. In small replicated plots and under ordinary farm seeding conditions over a large area, shrivelled seed sown in 1936 produced stands and yields which compared very well with those from normal seed. Spring conditions of that year were generally favourable and it is possible that under very unfavourable conditions the stands and yields from shrivelled seed might have been poorer. This is suggested by the results of the greenhouse tests.

SUMMARY

1. A direct examination and analysis of wheat kernels shrivelled by stem rust showed that the dry weights of the embryos was 41.6% of that of the embryos of the normal kernels, fibre 57.1%, and endosperm 37.0%. This indicates that the formation of endosperm tissue tends to be retarded by stem rust more than that of any other tissue in the kernels.

2. Shrivelled wheat germinated almost as well in the laboratory as normal wheat, but when planted in soil, it produced fewer and weaker plants. These weak plants emerged later than those from normal seed, had smaller root systems and were lighter in weight.

3. The low vigour of seedlings from shrivelled wheat was reflected in their poor resistance to common root rot which caused more seedling blight among the weak seedlings than among seedlings from normal seed. Greater permanent injury was done to these weak seedlings by severe top injury than to normal seedlings. However, these weak seedlings withstood freezing and prolonged drought as well as normal seedlings.

4. Treatment of shrivelled wheat with formalin caused serious reduction of germination and emergence and appeared to make seedlings very susceptible to common root rot. On the other hand, treatment with New Improved Ceresan and Leytosan was very slightly beneficial.

5. Despite the apparent weakness of plants from shrivelled wheat, good stands and yields were obtained from field tests of shrivelled wheat in 1936. The results of these tests are corroborated by the results of a provincial survey of areas where shrivelled wheat was sown.

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PRELIMINARY TESTS WITH SOME PLANT HORMONES IN THE ROOTING OF CUTTINGS OF CERTAIN FORAGE PLANTS[1]

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Preliminary tests were conducted to determine the possibilities of the use of hormones in propagating breeding material of forage plants in the greenhouse.

The hormones used were naphthyl-acetic and indolyl-acetic acids applied to cuttings of timothy (*Phleum pratense*), alfalfa (*Medicago media*), and red clover (*Trifolium pratense*).

TIMOTHY

Materials and Methods

The following substances were tested in the strengths indicated:

1. Powdered charcoal only.
2. 50 p.p.m. indolyl-acetic acid in powdered charcoal.
3. 100 " " " " "
4. 250 " " " " "
5. 1000 " " " " "
6. Powdered talc only.
7. 5 p.p.m. naphthyl-acetic acid in powdered talc.
8. 50 " " " " "
9. 100 " " " " "
10. 500 " " " " "
11. $\frac{1}{100}$ " " " a nutrient solution.
12. $\frac{1}{10}$ " " " " "
13. 1 " " " " "
14. 10 " " " " "
15. 100 " " " powdered charcoal.
16. Check (no treatment).

All the cuttings were planted in sand (crushed and washed sandstone) and fed a solution containing the following elements in parts per million: Mg 48.64, P 62.08, K 195.50, S 128.64, Ca 160.28, N 210.15, B 1.00 and Mn 0.19. Iron was added at the rate of 1.0 cc. of 0.5% solution $FeCl_3 . 6H_2O$ per litre of nutrient solution at each feeding. The sand was saturated with the above solution once a week and was washed down with distilled water once a week throughout the period of the experiment.

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First Test

Method

Cuttings were taken from an old sod which was dug up the last week in December and when the vegetative shoots were 4 to 6 inches in height. The cuttings were made at the surface of the ground and were trimmed away to leave 3 blades and a fully closed sheath about $2\frac{1}{2}$ inches in length. The cuttings were dipped into the different "dusts" and planted immediately. In treatments 11, 12, 13 and 14 the cuttings were planted directly into the sand which had been previously saturated with the required solutions. The pots were placed in greenhouse benches and received no direct sunlight.

During this test the temperature of the sand and air averaged as follows:

	8 A.M.	5 P.M.
Sand temperature	57.0	59.5
Air temperature	61.8	61.9

Results

The cuttings were treated on February 1st and by February 18 all the cuttings were apparently dead.

Second Test

Method

On the basis of the first test, it was decided that the temperature was too high. Consequently, a similar test was made of the same material with similar treatments as in the first test, but the pots were placed on the ground under the benches in the greenhouse.

The temperatures were as follows:

	8 A.M.	5 P.M.
Sand temperature	55.0	58.0
Air temperature	57.0	60.5

Results

The cuttings were treated on February 18, and on March 9 (19 days) the following observations were made:

Treatment No.	Percentage Living	Treatment No.	Percentage Living
1	—	9	10
2	10	10	—
3	10	11	—
4	20	12	—
5	—	13	—
6	—	14	10
7	—	15	30
8	20	16	20

Few of the living cuttings were callused while others showed short fine rootlets which were apparently quite normal.

The temperature maintained during this test was apparently still too high.

Third Test

Method

The former tests showed that the hormone treatments were not giving beneficial results, but the untreated plants were not growing satisfactorily either. The optimum temperature conditions were not yet established. It was decided to maintain the temperature as close to 50° F. as possible.

On March 9th, cuttings were made as in the two previous tests and the same treatments were applied. The mean temperatures were as follows:

	8 A.M.	5 P.M.
Sand temperature	49.2	51.9
Air temperature	50.0	53.2

Results

On April 5 (27 days) all the cuttings were examined, giving the following results:

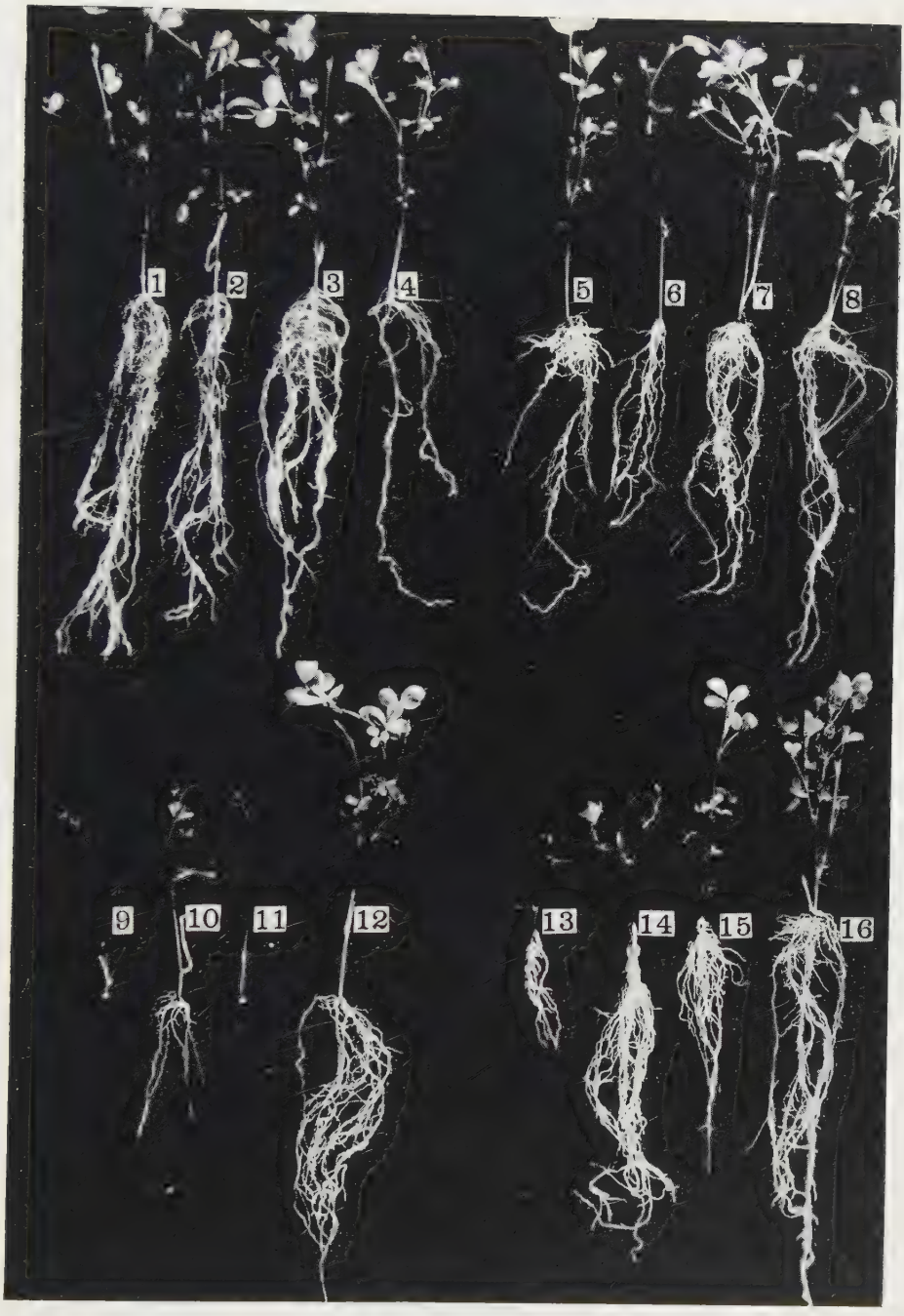
Treatment No.	Percentage rooted and living
2	16.6
3	16.6
4	33.2
8	33.2
9	16.6
14	16.6
15	83.0
16	33.2
All other treatments	0.0

It may be seen from the above, that with exception of treatment No. 15, (100 p.p.m. naphthyl-acetic acid in charcoal), the hormones failed to stimulate rooting in timothy.

PLATE I

PLATE I. Alfalfa cuttings treated February 3 and examined March 14. The cuttings received the following treatments:

1. 100 p.p.m. naphthyl-acetic acid in talc.
2. 50 p.p.m. " " " "
3. 5 p.p.m. " " " "
4. Talc only.
5. 1 p.p.m. naphthyl-acetic acid fed in nutrient solution.
6. 1/10 p.p.m. " " " " " "
7. 1/100 p.p.m. " " " " " "
8. 500 p.p.m. " " " " talc.
9. Check (no hormone).
10. " "
11. 100 p.p.m. naphthyl-acetic acid in charcoal dust.
12. 10 p.p.m. " " " fed in solution.
13. Dipped for 12hours in 100 p.p.m. naphthyl-acetic acid solution.
14. " " " " 100 p.p.m. " " " "
15. " " " " 50 p.p.m. " " " "
16. " " " " 5 p.p.m. " " " "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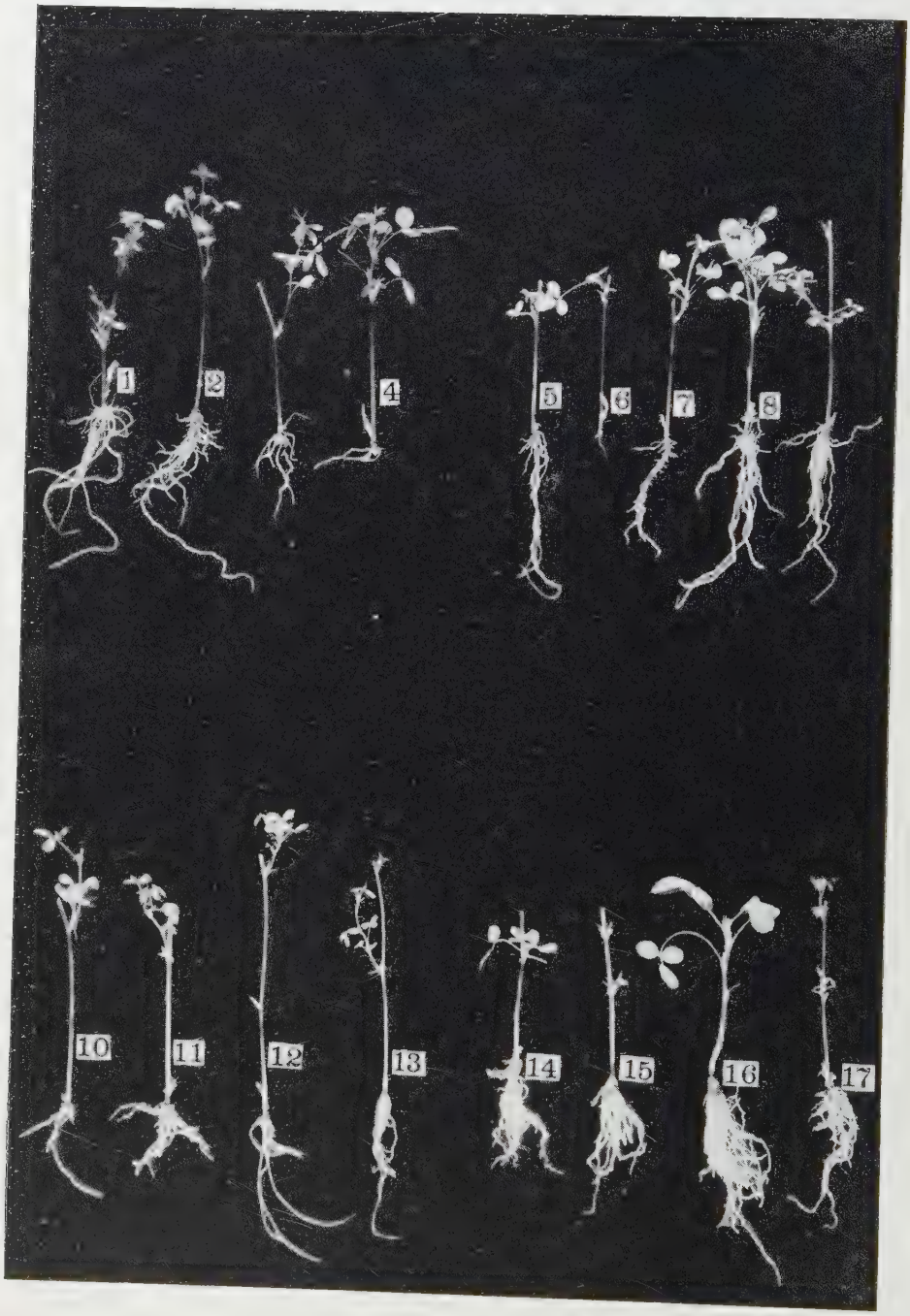
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General Discussion and Conclusion

From the results presented it may be seen that the effects of the treatments were influenced to some extent by the temperature. There were no treatments in this experiment that gave satisfactory results. This may have been due to factors such as: the degree of maturity of the tissue of the cuttings; temperature; light; humidity; the pH and chemical concentration of the medium in which they were planted; and finally the type and concentration of the hormone applied.

Further studies will have to be conducted before more definite conclusions can be made.

ALFALFA

Materials and Methods

Naphthyl-acetic acid was tested in different concentrations and treatments on alfalfa cuttings. All the cuttings were taken from a vigorous plant of alfalfa which was growing in the greenhouse for some time. These were taken by cutting the primary stems of the plant into 3-inch lengths, in such a way that each section had at least two nodes. The secondary branches were trimmed off so that the shoots arose from one node. The tips of the uppermost sections taken from the primary stems were removed, thereby making the cuttings all alike.

All the cuttings were planted in sand and fed a solution containing the following elements in parts per million: Mg 48.64, S 128.64, K 195.50, P 62.08, Ca 160.28, N 210.15, Mn 0.19, B 1.00 and Cu 0.127. Iron was added at the rate of 1.0 cc. of 0.5% solution $FeCl_3 . 6H_2O$ per litre of nutrient solution at each feeding. The sand was saturated with the above solution once a week and washed down with distilled water once a week throughout the period of the experiment.

PLATE II

PLATE II. Alfalfa cuttings treated February 21 and examined March 19. The cuttings received the following treatments:

1. 100 p.p.m. naphthyl-acetic acid in talc.
2. 50 p.p.m. " " " "
3. 5 p.p.m. " " " "
4. Talc only.
5. 1/10 p.p.m. naphthyl-acetic acid fed in nutrient solution.
6. 1/10 p.p.m. " " " " " "
7. 1/100 p.p.m. " " " " " "
8. 500 p.p.m. " " " " talc.
9. 500 p.p.m. " " " " "
10. Check (no hormone).
11. 100 p.p.m. naphthly-acetic acid in charcoal dust.
12. 10 p.p.m. " " " fed in nutrient solution.
13. 1 p.p.m. " " " " " "
14. Dipped for 12 hours in 100 p.p.m. naphthyl-acetic acid solution.
15. " " " " 50 p.p.m. " " " "
16. " " " " 50 p.p.m. " " " "
17. " " " " 5 p.p.m. " " " "

(Note that numbers 3 and 9 are missing from the Plate.)

First Test (Feb. 3 to Mar. 14) 39 days

The cuttings were dipped into naphthyl-acetic acid of different concentrations mixed with talc or charcoal as carriers, or fed the hormone along with the nutrient solution, or dipped for 12 hours into solutions of different concentrations and planted after the treatments.

Six cuttings constituted a unit under any particular treatment.

The treatments were as follows:

1. Talc only.
2. 5 p.p.m. naphthyl-acetic acid in talc.
3. 50 " " " "
4. 100 " " " "
5. 500 " " " "
6. $\frac{1}{100}$ " " " nutrient solution.
7. $\frac{1}{10}$ " " " " "
8. 1 " " " " "
9. 10 " " " " "
10. 100 " " " charcoal.
11. Check (no hormone).
12. 5 p.p.m. naphthyl-acetic acid—dipped 12 hours.
13. 50 " " " " "
14. 100 " " " " "
15. 500 " " " " "
16. 1000 " " " " "

The temperature and humidity records were taken with a thermohydrograph, but only the average data at 8 A.M. and 5 P.M. are given.

	8 A.M.	5 P.M.
Air temperature	60.8°	61.9°
Air humidity	77.5	78.1

Results

In general, very pronounced effects were observed. Some treatments stimulated the root development while in others the roots had a tendency to form a callus first. High concentrations killed the cuttings.

Detailed descriptions of the roots are given in Table 1.

It may seem that much better rooting was obtained by dipping the cuttings into naphthyl-acetic acid concentrations than by applying the same hormone in dry form. The best concentrations were 5 to 100 p.p.m. in talc (dust), and 1 p.p.m. fed in solution or dipped for 12 hours in 50 p.p.m. of naphthyl-acetic acid solution.

Second Test (Feb. 21 to Mar. 19) 26 days

On February 21, a second series of alfalfa cuttings was started. In this series 10 cuttings constituted a unit under any particular treatment.

The average temperature and humidity maintained during this test was a little higher being as follows:

	8 A.M.	5 P.M.
Air temperature	63.6°	62.4°
Air humidity	79.5	82.3

TABLE 1.—ALFALFA CUTTINGS TREATED WITH NAPHTHYL-ACETIC ACID. FIRST TEST, FEB. 3 TO MAR. 14 (39 DAYS)

Treatment no.	Hormone concentration and method of application	No. of cuttings tested	Results and remarks	Plate no.
1	Talc only (no hormone) dust	6	5 with fine long roots (2 have upward shoots); 1 with roots just starting	I—4
2	5 p.p.m. in talc dust	6	1 dead; 5 with good long roots from scar, new growth from upper nodes	I—3
3	50 p.p.m. in talc—dust	6	1 dead; 5 with fine roots from scar; 3 show callus at base	I—2
4	100 p.p.m. in talc dust	6	1 dead; 5 heavily rooted from scar; good growth from upper nodes	I—1
5	500 p.p.m. in talc dust	6	3 dead; 3 with fine long roots from scar and internode; good top growth	I—8
6	1/100 p.p.m. fed in solution	6	1 dead; 5 with long fine roots from scar; very good top growth and shoots from first node	I—7
7	1/10 p.p.m. fed in solution	6	2 dead; 4 with fine roots from scar; 1 has shoots from lower node; top not as healthy	I—6
8	1 p.p.m. fed in solution	6	1 dead; 2 with fine roots (better than No. 7); 3 just nicely started from scar (1 with new shoots)	I—5
9	10 p.p.m. fed in solution	6	2 dead; 3 with excellent roots and good new upward shoots; 1 small but nicely started	I—12
10	100 p.p.m. in charcoal dust	6	2 dead; 1 with good long roots; 3 very poor, with callus and few small roots	I—11
11	Check—(no hormone)	6	1 dead; 1 with good roots not very long; 1 definite callus; 3 spindly growth, few roots	I—9 and 10
12	5 p.p.m. in solution—dipped for 12 hrs.	6	1 dead; 5 with very heavy rooting system, shoots arising at lower scar and node	I—16
13	50 p.p.m. in solution—dipped for 12 hrs.	6	0 dead; 1 with lower end rotted but roots arise above; rest heavily matted with roots	I—15
14	100 p.p.m. in solution—dipped for 12 hrs.	6	1 dead; 3 with lower end rotted but roots arise above this; all have roots arising between nodes	I—13 and 14
15	500 p.p.m. in solution—dipped for 12 hrs.	6	6 dead	
16	1000 p.p.m. in solution—dipped for 12 hrs.	6	6 dead	

The duration of the experiment was 26 days as compared with 39 days in the previous test, and for this reason the mass of roots developed by the cuttings is much smaller, as may be seen by comparing Plates 1 and II.

Results

The results in detail are given in Table 2.

It may be seen from the data presented and from the plates that there was less top growth developed and the root systems were not as extensive as in the first series. Some cuttings failed to show roots at the end of 26 days but remained quite green and healthy. Again 5 p.p.m. was about the optimum concentration and better results were obtained by soaking the lower part of the cuttings for 12 hours in the solution of naphthyl-acetic acid.

RED CLOVER

One series of red clover cuttings was started on March 15, and examined on April 5. The treatments were almost identical with those used for alfalfa. All cuttings after the application of different hormones were planted in sand and fed a similar solution to the alfalfa cuttings above. The average temperature and humidity is given below:

	8 A.M.	5 P.M.
Air temperature	64.5°	65.2°
Air humidity	82.5	83.5

Results

Table 3 shows that a few cuttings died at low as well as at high concentrations of the hormone. It was seen that by dipping the cuttings into solutions of naphthyl-acetic acid for 12 hours the rooting was very extensive but seemed to be forced too much. The rootlets were white, brittle, short and usually flat, arising in bunches along the stem. Compare Nos. 12–15 with other red clover cuttings in Plate III.

The treatment of 10 p.p.m. naphthyl-acetic acid fed in solution seemed to be the best of the treatments nos. 6 to 9.

PLATE III

PLATE III.—Red clover cuttings treated March 15 and examined April 5. The cuttings received the following treatments:

1. 100 p.p.m. naphthyl-acetic acid in talc.
2. 50 p.p.m. " " " "
3. 5 p.p.m. " " " "
4. Talc only.
5. 1/10 p.p.m. naphthyl-acetic acid fed in nutrient solution.
6. 1/100 p.p.m. " " " " " "
7. 500 p.p.m. " " " in talc.
8. Check (no hormone).
9. 100 p.p.m. naphthyl-acetic acid in charcoal dust.
10. 10 p.p.m. " " " fed in nutrient solution.
11. 1 p.p.m. " " " " " "
12. Dipped for 12 hours in 75 p.p.m. naphthyl-acetic acid solution.
13. " " " " 100 p.p.m. " " " "
14. " " " " 50 p.p.m. " " " "
15. " " " " 5 p.p.m. " " " "

TABLE 2.—ALFALFA CUTTINGS TREATED WITH NAPHTHYL-ACETIC ACID. SECOND TEST, FEB. 21 TO MAR. 19 (26 DAYS)

Treatment no.	Hormone concentration and method of application	No. of cuttings tested	Results and remarks	Plate no
1	Same as in first test	10	0 dead; 5 with fine roots from scar; 2 bases rotted off, few roots; 3 green, no roots	II—4
2	Same as in first test	10	1 dead; 1 green but no roots; 8 with fine roots, better than No. 1	II—3
3	Same as in first test	10	1 dead; 9 nicely rooted, the roots are more numerous and matted than No. 2	II—2
4	Same as in first test	10	0 dead; 9 well rooted, the rootlets thicker than No. 3; 1 with callus only	II—1
5	Same as in first test	10	0 dead; 10 well rooted, the rootlets not long but heavily matted from scar and node	II—8 and 9
6	Same as in first test	10	3 dead; 7 rooted, but rootlets fine and not very numerous	II—7
7	Same as in first test	10	2 nearly dead; 2 green but no roots; 6 rooted little better than No. 6	II—5 and 6
8	Same as in first test	10	1 dead; 3 green but no roots; 6 well rooted, rootlets matted at base	II—13
9	Same as in first test	10	2 dead; 2 green but no roots; 6 rooted about the same as in No. 8	II—12
10	Same as in first test	10	1 dead; 8 heavily rooted from lower scar; 1 with callus only	II—11
11	Same as in first test	10	2 dead; 1 green but no roots; 7 with small roots but look sturdy	II—10
12	Same as in first test	10	2 dead; 1 green but no roots; 7 very well rooted, mostly from lower scar	II—17
13	Same as in first test	10	1 dead; 9 with an enormous mass of roots—they are quite brittle	II—15 and 16
14	Same as in first test	10	4 dead; 2 with roots just starting to show; 4 with numerous roots along stem	II—14
15	Same as in first test	10	9 dead; 1 green but no roots showing, the end of cutting rotten	
16	Same as in first test	10	10 dead	

TABLE 3.—RED CLOVER CUTTINGS TREATED WITH NAPHTHYL-ACETIC ACID. MARCH 15 TO APRIL 5 (21 DAYS)

Treatment No.	Hormone concentration and method of application	No. of cuttings tested	Results and Remarks	Plate no.
1	Talc only (no hormone) dust	6	2 dead; 1 green but no roots; 2 rooted at first node only; 1 with excellent roots.	III—4
2	5 p.p.m. in talc—dust	6	1 dead; 2 green but no roots; 1 rooted at node and grew from there, original cutting died; 2 excellent	III—3
3	50 p.p.m. in talc—dust	6	0 dead; 1 green but no roots; 2 rooted at 1st node only; 3 well rooted at scar and node	III—2
4	100 p.p.m. in talc—dust	6	0 dead; 1 green but no roots; 1 few rootlets at scar; 4 nicely rooted at node and little at scar	III—1
5	500 p.p.m. in talc—dust	6	0 dead; 3 extremely well rooted at scar only; 3 well rooted at node and little at scar	III—7
6	1/100 p.p.m. fed in solution	6	1 dead; 1 green but no roots; 1 rooted at node then died; 3 rooted at node and little at scar	III—6
7	1/10 p.p.m. fed in solution	6	1 dead; 2 green but no roots; 1 rooted at node then died; 2 well rooted at node also at scar	III—5
8	1 p.p.m. fed in solution	7	1 rooted at scar only then died; 3 green but no roots; 3 well rooted at node and little at scar	III—11
9	10 p.p.m. fed in solution	6	4 rooted at node and to some extent at scar; 2 rooted at node only	III—10
10	100 p.p.m. in charcoal—dust	6	1 dead; 2 green but no roots; 1 with very small rootlets at scar; 1 very well rooted at scar; 1 at scar and node	III—9
11	Check (no hormone)	6	1 dead; 1 rooted at scar only; 1 rooted at node only; 3 with fine roots at node and scar	III—8
12	5 p.p.m. in solution dipped 12 hrs.	6	2 green but no roots; 4 well rooted at scar only, rootlets are very brittle rather short and forced	III—15
13	50 p.p.m. in solution dipped 12 hrs.	6	2 green but no roots; 1 with few roots at scar only; 3 well rooted at scar and node—similar to No. 12	III—14
14	100 p.p.m. in solution dipped 12 hrs.	6	2 green but no roots; 1 rooted at scar only: 3 well rooted at scar and node, roots finer than No. 12	III—13
15	500 p.p.m. in solution dipped 12 hrs.	6	6 dead	
16	75 p.p.m. in solution dipped 12 hrs.	6	1 dead; 1 green but no roots; 1 rooted at scar only; 3 well rooted at scar and node	III—12

Of the dip treatments (nos. 12 to 16) a solution of 50 p.p.m. of naphthyl-acetic acid seemed best.

Conclusions on Treatment of Cuttings of Alfalfa and Red Clover

It appears that temperature and humidity are important factors.

A concentration of 50 p.p.m. of naphthyl-acetic acid in talc applied to the fresh scar of the cuttings appeared to be quite satisfactory. Where a greater mass of roots is required, it is better to soak the basal ends of the cuttings in a solution of 5 to 50 p.p.m. naphthyl-acetic acid for a period of not more than 12 hours.

Further experiments are necessary to observe the growth and behaviour of the cuttings which have been rooted by different hormone treatments. It is believed that such large masses of roots as produced by some treatments are not necessary.

CONCLUSIONS

(1) The plant hormones tested are of value to the plant breeder in propagating valuable strains of certain forage plants.

(2) The results obtained indicate that the effects of the treatments are influenced to some extent by temperature, humidity and light in the greenhouse; by the degree of maturity of the tissue of the cuttings; by the pH and chemical concentration of the medium in which they are planted; and finally by the type and concentration of the hormone applied.

(3) There were no treatments in this experiment that gave satisfactory results in rooting timothy (*Phleum pratense*) cuttings.

(4) The optimum treatments for alfalfa (*Medicago media*) and red clover (*Trifolium pratense*) were approximately any one of the following with naphthyl-acetic acid:

(a) 50 p.p.m. in talc applied to the fresh scar of the cutting.

(b) basal ends of cuttings dipped for 12 hours in 5–50 p.p.m. solution.

(c) 10 p.p.m. fed in solution in nutrient culture solution.

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BOOK REVIEW

KUBIENA, WALTER A. Micropedology. Collegiate Press, Inc., Ames, Iowa, 1938. Pp. xvi+243; illus. $3.00.

This is a book of the pioneering type, in which Dr. Kubiena calls attention to a new concept in soil study which promises to have an important bearing on fundamental soil research. In the author's opinion, one with which most of us will agree, pedology should be regarded as an independent science, "the basis of agricultural soil science just as geology as an independent science is the basis of mining". The development of pedology, the objective study of the soil, is but in its early stages. Hitherto most of our knowledge of the soil is derived from examination of the soil "*en masse*", with little regard to its minute structural characteristics, which are so easily obliterated in the handling the soil receives in the course of our general physical, chemical and biological examination.

Knowledge of the soil with respect to the details of its structural entity is regarded as an essential part of pedology as a whole, and the author considers the adaptation of microscopic methods as a necessary complement to, and by no means a substitute for, the existing methods and procedures for soil research. Just as petrography, previously concerned with rock profiles and fragments, received a stimulus some seventy or eighty years ago through the introduction of microscopic methods for rock examination, so pedology as a science should receive an equal stimulus by application of microscopic procedures for the study of soil fabrics and soil dynamics.

The book is concerned with the basic principles of microscopic pedology in which the author himself has contributed largely to the development of methods and procedures. The subject matter is presented in four main Parts. Part I (General) outlines the principles of micropedology and the use and development of micro-technique in other natural sciences. Part II (The Technique of Micropedology) includes chapters on incident light microscopes, the soil microscope, performance of micro-manipulations, microscopic field investigations, soil sampling, soil preparations, fabric reactions, optical methods and microchemical methods. Part III (Soil Fabrics) treats of the arrangement of the constituents of the soil and their role in relation to each other, and is particularly illuminating in illustrating the special scope of micropedology. As with other sections of the book, the inclusion of many original photomicrographs and other figures adds greatly to the value of the textual matter of this part. Part IV. (Biological Soil Microscopy) treats of the characteristics of the micro-habitat, the relationship of soil fabrics and soil biology, observations on humus formation, and the application of direct microscopy to the study of the soil microflora and microfauna. Soil microbiologists should find this part of special interest, particularly the discussions on the associations, activity and morphology of the micro-organisms as influenced by space conditions, the microclimate, the pH, salt concentrations and food conditions in the micro-habitat.

Every student of soil science will read this book with interest and profit. It is important not only on account of its originality of outlook and the development of new technique, but also from the point of view of the possible applications of the fundamental principles involved to the varied problems of practical soil science.

—A. G. LOCHHEAD.